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Archives of Internal Medicine

VOLUME 46 SEPTEMBER, 1930 NUMBER 3

DIGESTION

EFFICIENCY WITH VARIOUS FOODS AND UNDER VARIOUS CONDITIONS *

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The studies here reported represent a continuation and an amplification of those published by Hosoi, Alvarez and Mann.[1] We wondered if we could improve intestinal absorption by dividing the food into a number of portions and giving the portions at intervals, and we thought it might be helpful to mince the food or to slow the progress of peristalsis with morphine. We wondered also what would be the effect of giving progressively larger amounts of foods. Would the efficiency of digestion fail gradually at a certain point or would it break suddenly?

METHOD OF EXPERIMENTATION

As was reported by Hosoi, Alvarez and Mann, the colon was removed from three dogs, and the distal end of the ileum was joined to the stump of the rectum. In this way digestion in the small intestine could be studied in animals that were free from the soiling and irritation of the skin which is inevitable with an ileal fistula. These dogs, after being used for the experiments already reported, were kept about the laboratory for almost a year. It was then found, with the help of the roentgen ray, that some hypertrophy had taken place in the rectal segment. This was most marked in one animal (the third dog) in which at operation more of the rectum had to be left than in the others. This hypertrophy of the rectal segment probably accounts for the fact that with certain foods the fecal residues of these dogs are somewhat different now from what they were a year ago. As one would expect, the largest difference was observed in the third dog, in which there is now an almost normal rectum.

* Submitted for publication, Feb. 28, 1930.

* From the Division of Medicine, the Mayo Clinic and the Division of Experimental Surgery and Pathology, the Mayo Foundation.

1. Hosoi, Kiyoshi; Alvarez, W. C., and Mann, F. C.: Intestinal Absorption: A Search for a Low Residue Diet, Arch. Int. Med. **41**:112 (Jan.) 1928.

The accompanying table shows, for the first three dogs, the difference in the amount of feces (formed during a twenty-four-hour fast) obtained by Hosoi in 1927 and by us a year later when the remnant of colon had hypertrophied. It shows how much of the fasting fecal material is contributed by the colon. It is worth noting, however, that even in the animals with a small remnant of colon the discharges had almost no fecal odor.

At the beginning of this study three new dogs were operated on. In one, almost complete colectomy was done, while in the other two, ileostomy was performed a short distance above the ileocecal sphincter. The animal from which the colon was removed was a poodle weighing 4.9 Kg.; one dog was a fox terrier which weighed 7.6 Kg., and the other was a poodle which weighed 6.2 Kg.

In order to collect the fecal residue from the animals with an ileal fistula, an attempt was first made to strap a cup over the opening, but this apparatus did not work well and it had to be abandoned. The

Moist Weight of Discharges Recovered During a Twenty-Four-Hour Period of Fasting

Dog	A Few Weeks After Operation, Gm.	Ten Months After Operation, Gm.
First	6.8	8.3
Second	9.9	24.0
Third	15.6	42.0

dog was kept loosely restrained in a wooden frame, in which he usually slept for the eight-hour period of observation. A receptacle placed on the floor under the fistula caught everything that was passed. The other dog with the fistula was easier to handle when kept in a metabolism cage.

The dogs on which colectomy had been done were easier to work with than those with ileostomy. They were cleaner and more easily cared for, and it was easier to keep them in a good state of nourishment. There was little difference in the amount of fecal residue obtained from the dogs with the two types of operation.

It was found that in the three dogs originally used by Hosoi, feces could no longer be removed easily with the help of the tube which he devised; so these dogs also were kept in metabolism cages, and at the end of every hour the material passed was collected from the pan.

The dogs were fed weighed amounts of food at 8:30 a. m. Some of the less palatable foods had to be given by stomach tube. As the feces were passed, they were weighed moist, and the figures were plotted as cumulated percentages of the amounts fed. Water was not given during the eight-hour period of observation. When we were studying food which we knew would not be digested for a long time, the dog was made

to fast for twenty-four hours after the meal, so that when the next experiment started the bowel would surely be empty.

In all six dogs it was found that the taking of food was followed in a few minutes by the extrusion of a small amount of material from the bowel. This movement was probably brought about by the so-called ileocolic reflex which has been described by a number of writers. In another half hour there was generally another small movement which consisted of residues of the meal from the day before. In the dogs with an ileostomy opening, the next bowel movement usually occurred about two and a half hours after the taking of food, and in those which the colon was excised, the interval more often was from three to five hours. When liquid food was taken, this interval was usually somewhat shortened. After this, the fecal residue continued to appear at fairly frequent intervals for six hours longer. Movements were seldom seen after nine hours. On one occasion it was noted that it took seventeen hours for a chicken bone to pass.

In these experiments we have used as an index to the efficiency of digestion the percentage relationship between the moist weight of the feces and the moist weight of the food. It would have been more satisfying to deal with dry weights, but the work of Hosoi, Alvarez, and Mann revealed so little difference in the water content of feces obtained with different diets that, for the purposes of this study, we thought it hardly necessary to go to the extra labor of drying the foods and the residues.

EXPERIMENTS WITH DIFFERENT FOODS

Milk.—We confirmed Hosoi's observation that most of the residue from milk is passed within a few hours. Large amounts of milk were given in an attempt to overwhelm the digestive tract. Sometimes we gave as much as 90 Gm. for each kilogram of body weight. In a few instances undigested curds were passed, but there was never any change in the type of curve obtained, or any increase in the percentage of residue excreted.

Sour milk was better digested than sweet milk. The residues from the former amounted usually to from 18 to 25 per cent and from the latter to from 33 to 54 per cent.

Hosoi found that the addition of bread to milk tended to decrease the percentage of moist residue obtained. These results were confirmed, and it was found that other foods such as meat, raw egg, potato, and Karo syrup, when added to milk, also tended to decrease the amount of residue obtained. Similarly, the residue obtained after feeding bread was often diminished by the addition of milk to the ration, even when large amounts of the fluid were used. There was no reduction, however, when evaporated milk was added; this somewhat increased the amount of residue. The weights of the residue from meat and potato were not materially altered by the simultaneous giving of milk.

Lactose.—Hosoi called attention to the purgative action of lactose. When added to farina it increased the amount of residue obtained. These experiments were repeated and the observations confirmed (chart 1). Lactose was given in a 25 per cent solution and also in the form of

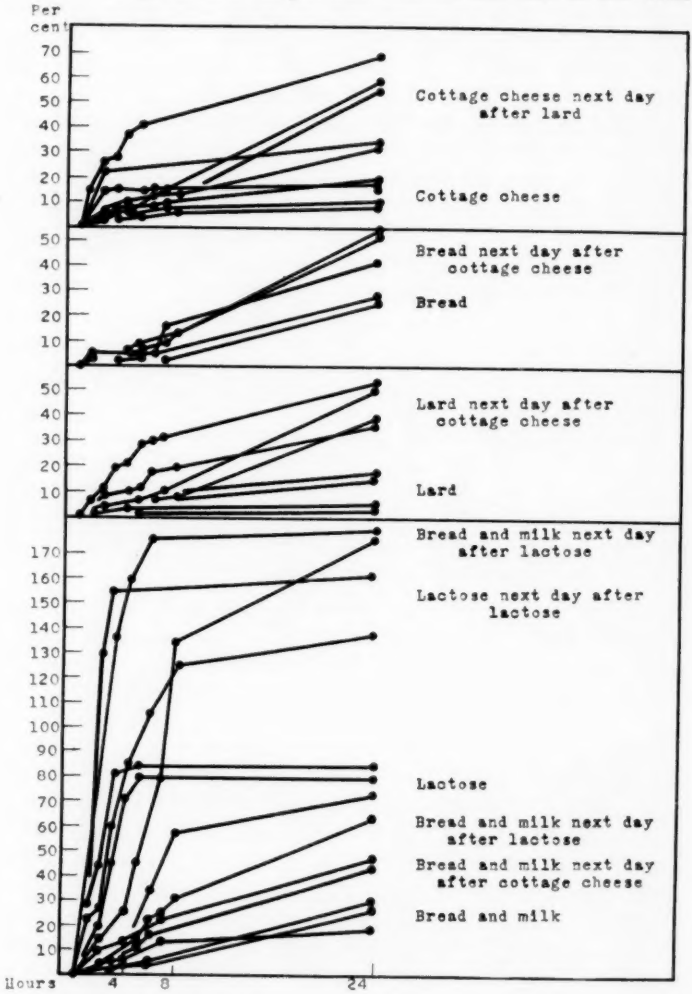

Chart 1.—In this and all subsequent illustrations abscissas represent hours after feeding and ordinates represent amounts of residue expressed as cumulated percentages of amounts of food given. Lines connect data obtained in one experiment. Data presented in this chart show that certain foods given one day can influence adversely the digestion of others given the following day.

powder. The substance had a cathartic effect, and when it was given repeatedly on successive days, the bowel became increasingly irritable. On days following the administration of lactose, any food that was given went through the bowel more rapidly than normal and the amount digested was lessened. The animals did not seem to feel well, and they showed little inclination to eat. These observations agree with those of Mitchell [2] and Evans and Burr [3] who found that rats go downhill rapidly when given considerable amounts of lactose.

Cheese.—Hosoi found that cottage cheese was well digested, whereas grated Swiss cheese in amounts of 26.4 Gm. for each kilogram of body weight was hardly digested at all. We found that Swiss cheese was well digested when it was given in the form of lumps; the residues then never represented more than 32 per cent of the weight of the original ration. At times the percentage was as low as 14 (chart 2).

Cottage cheese continued to be well digested even when given in amounts as large as 102 Gm. for each kilogram of body weight. We were unable with this substance to produce a break in the efficiency of digestion. The residue came through somewhat faster than did those from Swiss cheese.

Cottage cheese given one day always impaired the digestion of food given the following day. Charts 1 and 3 show examples of such effects. Similar deleterious effects were observed after the giving of lard and of lactose.

Meat.—It has been shown by Cannon [4] that coarse hard particles of food are slow to leave the stomach. It might well be, then, that the digestion of food such as meat, which needs to be broken up first by pepsin, could be improved by being given in the form of lumps which would be retained for some time in the stomach. One might spare the stomach by giving the meat in the form of hamburger steak, but the greater strain put on digestion in the bowel might prove to be too much for it. Actually, Cohnheim [5] and later Hosoi found that meat is best digested when given in the form of lumps. We have again found this to be true (chart 2), and it now appears that the dog is following a good instinct when he bolts his food unchewed.

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2. Mitchell, Helen S.: Comparative Physiological Values of Five Carbohydrates, Based on Growth and Fecal Analysis, Am. J. Physiol. **79**:537 (Feb.) 1927.

3. Evans, H. M., and Burr, G. O.: A New Dietary Deficiency with Highly Purified Diets, Proc. Soc. Exper. Biol. & Med. **24**:740, 1927.

4. Cannon, W. B.: The Mechanical Factors of Digestion, London, Edward Arnold, 1911, pp. 227.

5. Cohnheim, Otto: Beobachtungen über Magenverdauung, München. med. Wchnschr. **2**:2581 (Dec. 24) 1907.

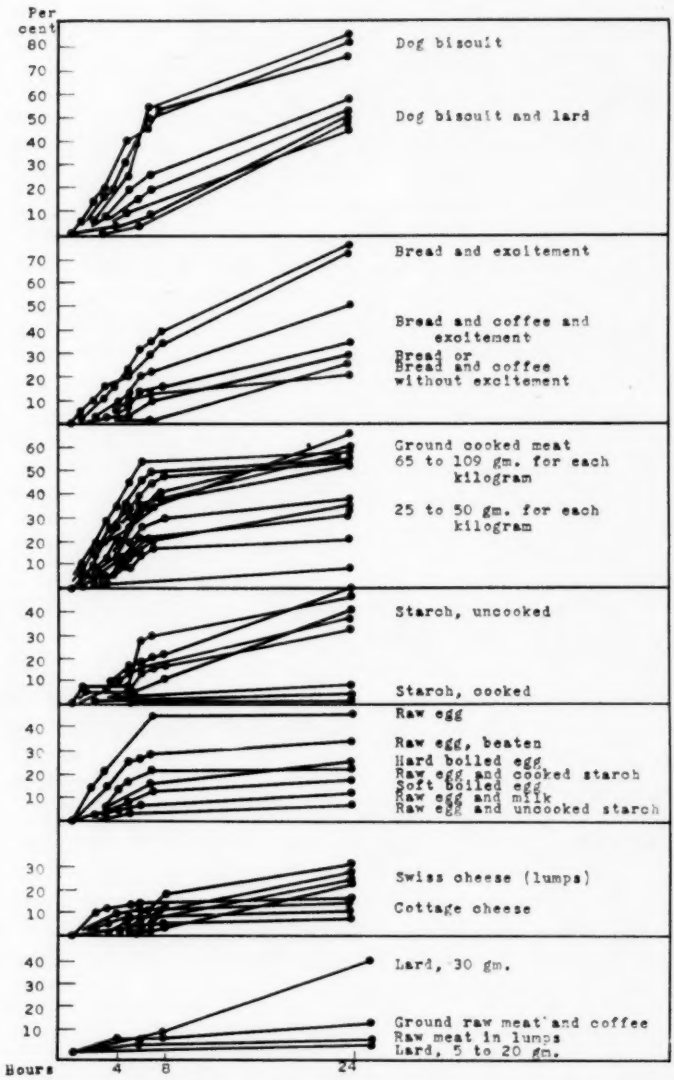

Chart 2.—The effect is shown of adding one food to another, the effect of excitement, the effect of giving large quantities of food, the effect of cooking of food, the difference in the digestibility of two types of cheese and the effect of hashing the food.

We have given raw meat, in the form of lumps, in amounts as large as 175 Gm. for each kilogram of body weight, and have marveled at the thoroughness with which it was digested. Raw meat was handled better than cooked meat. It seemed to be impossible to break down the ability of the stomach and bowel to handle these large amounts of food, and at no point was there any sudden increase in the percentage of residue excreted. The result of the experiments was different, however, when the meat was given finely ground. In this case, a large increase in the amount fed resulted in an increase in the percentage of undigested residue.

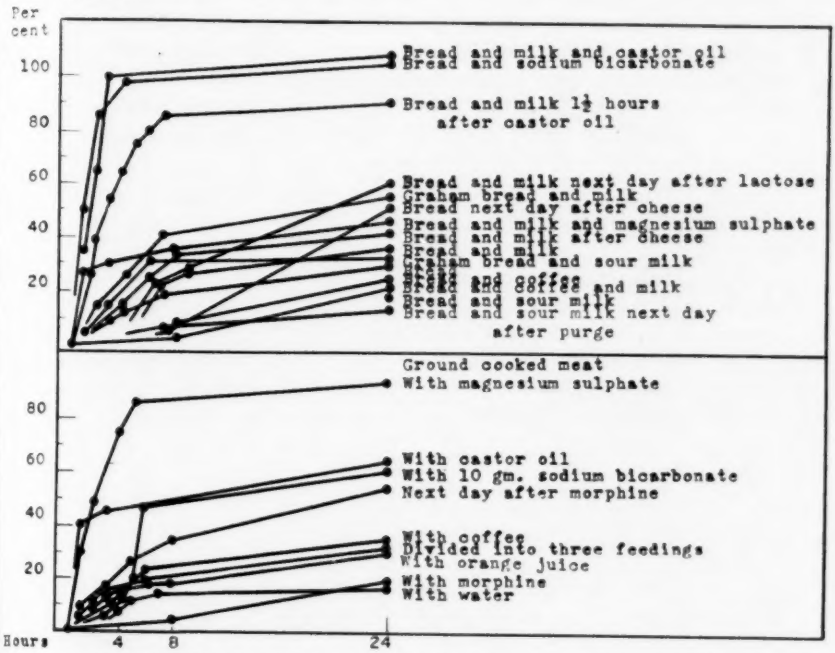

Chart 3.—The digestibility of bread when eaten alone, when eaten with other foods, or after drugs were taken is shown; also the digestibility of meat under different conditions.

Fat.—Hosoi found that with his dogs he could easily reach the limits of digestion of fat. Lard or butter, when given in considerable quantities, always produced large foul-smelling stools. In the experiments here reported, 10 Gm. for each kilogram of body weight appeared to be about as much as the dogs could stand (chart 2). More than this gave rise to vomiting and diarrhea. On a few occasions, however, when the dogs succeeded in retaining lard in amounts as high as 30 Gm. for each kilogram of body weight, it was well digested.

Cannon observed, years ago, that the mixing of fat with protein or carbohydrate would cause these two foodstuffs to leave the stomach more slowly than they otherwise would. In the experiments here reported a dog biscuit containing bone-meal, flour and chopped meat was first tested, and it was found to leave a residue of from 80 to 87 per cent. Lard was then mixed with, or melted into, the broken biscuit, and the residue was found to have decreased to about 50 per cent (chart 2). Possibly the fat caused the biscuit to stay long enough in the stomach so that more of it could be attacked by the gastric juice.

Eggs.—Hosoi confirmed what has been known for some time—that raw egg albumin runs through the digestive tract so rapidly that much of it reaches the ileocecal sphincter unchanged. Soft-boiled and hard-boiled eggs were digested much better. Rose and MacLeod [6] found that raw egg white was better utilized after it was beaten up with air, and this we have confirmed (chart 2). In our experiments, raw whole egg left a residue of from 35 to 60 per cent. Soft-boiled eggs left the smallest residue. Raw egg produced foul, foamy, liquid discharges in which the egg albumin was so little altered that the dogs sometimes ate it again.

The addition to raw egg of raw starch which is not digested by the dog, and which greatly slows the progress of material through the intestine appeared to improve the digestion of the egg. Whereas, the residue from raw egg amounted to from 25 to 36 per cent and that from raw starch amounted to 45 per cent, the residue from both together was usually between 17 and 25 per cent, and on one occasion it was only 7 per cent.

The addition of milk to raw egg resulted in a mixture which was better digested than milk alone. The popular use of egg-nog, without alcohol, would therefore appear to be well justified.

Bread.—Hosoi found that the residue from white bread amounted to from 44 to 52 per cent. In our experiments the results were considerably better, and the percentage varied between 26 and 41 (chart 3). As was to be expected, graham bread left considerably larger residues than did white bread. Strange to say, these residues were decreased in amount when milk or coffee was given with the bread. This improvement in digestion was more marked with sour milk than with sweet milk.

Starch.—Raw cornstarch given in a 33 per cent watery suspension gave fecal residues varying between 36 and 50 per cent (chart 2). Our impression was that practically none of this starch was digested because both macroscopically and microscopically the material recovered appeared

6. Rose, Mary S., and MacLeod, Grace: Some Human Digestion Experiments with Raw White of Egg, J. Biol. Chem. **50**:83 (Jan.) 1922.

to be unchanged. Strange to say, it passed through the digestive tract slowly, requiring more than twenty-four hours. It probably was caught here and there in the valvulae conniventes.

Dextrose.—As Hosoi showed, dextrose and sucrose are completely or almost completely absorbed. The efficiency of digestion depends somewhat on the concentration of the material given. In one of our experiments in which a concentrated solution of dextrose was administered, it produced such severe vomiting that a lump of formed feces was finally brought up. In only about half of the experiments could the dogs retain a 50 per cent solution of dextrose given in amounts representing about 40 cc. for each kilogram of body weight. When they did retain it, all the sugar was apparently absorbed, because the residues were about the size of those that one would get from a fasting bowel. Strange to say, chocolate candy with cream centers left a residue of from 25 to 50 per cent.

The mixture of Karo syrup and milk which is used in the laboratory for the feeding of animals after operation was well utilized. From 17 to 27 per cent appeared in the feces, as compared with from 35 to 50 per cent for milk alone.

Potatoes, Stewed Tomatoes, Baked Bananas, Stewed Prunes, Stewed Corn and Beets.—These foods were all poorly digested and gave residues varying in amount from 30 to 75 per cent. Digestion could be improved somewhat by the addition of gravy or milk to the foods. Potatoes were better digested when given in lumps than when given mashed.

Baked bananas left a residue which was remarkably constant in its amount, about 37 per cent. Our observations, therefore, do not support the statements of those investigators who have made the claim that cooked banana is a highly digestible food. In Hosoi's experience raw banana was the most poorly digested of all the foods studied.

Rice appeared to be almost perfectly digested. Peptone was also well digested and absorbed.

Fruit Juices.—The dogs were given mixtures of equal parts of orange juice or lemon juice and water, either alone or with meat. The addition of a little meat caused the dogs to drink the liquid voluntarily. The orange juice gave a residue of from 11 to 13 per cent. When added to meat, the amount of residue usually obtained with this food was not increased. The giving of lemon juice resulted in an excretion of residues amounting to from 20 to 55 per cent. It is probable that acids increase the rate of passage of material through the bowel, and this would account for the poorer digestion observed with lemon juice.

Coffee.—Coffee when given with bread or meat appeared to be as well absorbed as water (chart 3). The stools tended, however, to become loose.

Desserts.—Miller, Fowler, Bergeim, Rehfuss and Hawk,[7] who studied the rate of emptying of the stomach in normal students, concluded that, so far as gastric digestion is concerned, pies with crusts are not particularly harmful. Pudding left the stomach a little faster than pie, and pie left faster than cake. In one dog in which complete colectomy had been performed, custard pie gave a residue of 28 per cent; cornstarch pie, 36 per cent; pie crust, 48 per cent; apple pie, 55 per cent and prune pie, 74 per cent.

INFLUENCE OF VARIOUS FACTORS ON DIGESTION

Forced Feeding.—There have been a number of observations in the past which have led experimenters to believe that food eaten freely with appetite will leave the stomach more rapidly than food given by stomach tube. We, however, have been unable to detect any difference in the amount of fecal residue obtained when foods were given in these two ways.

Anxiety and Excitement.—As has been pointed out in a recent review by Alvarez,[8] anxiety and excitement tend to retard digestion, and evidences of this were noticed during the progress of the studies reported here. One of the dogs, a nervous, restless animal, fretted a good deal when restrained in the type of sling in which his fellows dozed. Under these circumstances, the fecal residue from bread and butter was about 77 per cent of the amount ingested. Later when he lay quietly in a metabolism cage, the percentage dropped to 41 per cent (chart 2). The other dog with a fistula, that was quiet and well behaved, digested just as well when in the sling as when in a cage.

Heat and Cold.—It has been thought by some writers that the temperature of the food might influence the completeness of digestion, but Hawk[9] and his associates could not detect any such effect and neither could we.

Several Small Feedings as Compared With One Large Feeding.—Hosoi, Alvarez, and Mann suggested that better absorption of foodstuffs might be secured if small amounts were given at short intervals, and

7. Miller, R. J.; Fowler, H. L.; Bergeim, Olaf; Rehfuss, M. E., and Hawk, P. B.: The Gastric Response to Foods. XII. The Response of the Normal Human Stomach to Pies, Cakes and Puddings, Am. J. Physiol. **52**:248 (June) 1920.

8. Alvarez, W. C.: Ways in Which Emotion Can Affect the Digestive Tract, J. A. M. A. **92**:1231 (April 13) 1929.

9. Miller, R. J.; Bergeim, Olaf; Rehfuss, M. E., and Hawk, P. B.: Gastric Response to Foods. XI. The Influence of Tea, Coffee and Cocoa upon Digestion, Am. J. Physiol. **52**:28 (May) 1920.

Bateman [10] found that the diarrhea produced by raw white of egg was less severe when the substance was given in divided doses.

Chart 4 shows that when a quantity of food was divided into fractions which were given to the dog at hourly or half-hourly intervals, the residues appeared more promptly than when the total amount was eaten at one time and the percentage undigested was larger. This can be explained by the fact that each successive feeding stimulates peristalsis and gives rise to rush waves which carry the food rapidly down the bowel.

Sodium Bicarbonate.—Ten grams of sodium bicarbonate added to meat or bread increased the amount of residue obtained and increased

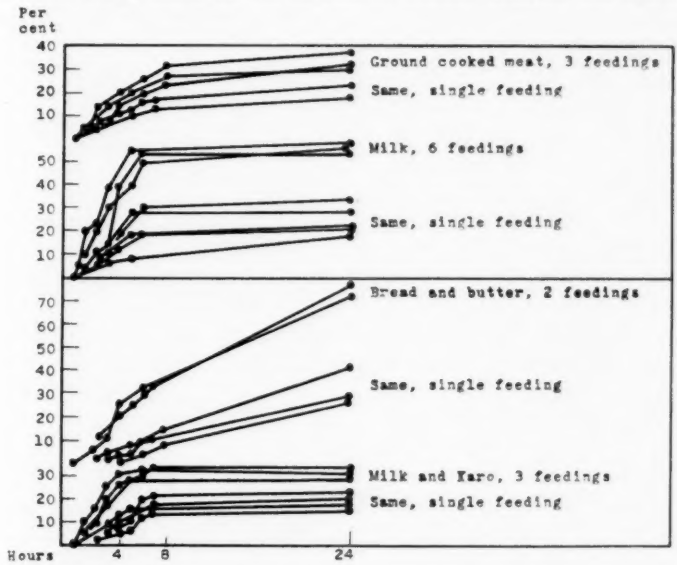

Chart 4.—Difference in digestibility when food was taken in one meal or divided into several portions and eaten at brief intervals.

the content of moisture (chart 3). The dogs became thirsty. The residues were alkaline except in those experiments in which the drug was added to bread and sour milk.

Purgation.—The giving of 30 cc. of castor oil or 17 Gm. of magnesium sulphate two hours before the giving of food always increased the amount of residue obtained. The giving of the purge twenty-four hours before a feeding usually increased the amount of residue obtained from the food (chart 3).

10. Bateman, W. G.: The Digestibility and Utilization of Egg Proteins, J. Biol. Chem. **26**:263 (Aug.) 1916.

Barium Sulphate.—Alvarez and Freedlander [11] suggested that the addition of a large amount of indigestible material such as barium sulphate to a meal might act as a laxative and might increase the rate of progress of material through the digestive tube. Some of the dogs were given condensed milk with varying amounts of barium sulphate and acacia. Under these conditions, the excretion of residues from milk was found to be more rapid and the amounts obtained were larger.

Morphine.—Plant and Miller,[12] who studied the effect of morphine on dogs with a Thiry-Vella fistula, found that it increased the tonus and peristaltic activity of the bowel and lessened the tonus of the gastric musculature. They concluded that the giving of morphine resulted in more complete digestion, because the food stayed a longer time in the stomach.

In the experiments reported here the dogs were given canned ground horse-meat and thirty minutes later were given, hypodermically, from 0.03 to 0.06 Gm. of morphine sulphate. Vomiting often occurred, and the animals remained somnolent for about eight hours. During this time practically no residue appeared. During the night, residue amounting to about 20 per cent was passed, and during the following day more was obtained (chart 3). In some of the animals an increase in intestinal tonus immediately after the giving of morphine showed itself in a sudden spurting of intestinal contents from the ileal fistula.

Reaction of the Intestinal Contents.—The residue was usually alkaline to litmus, but with some foods it was acid. It was often acid after the giving of lard, milk and lard, milk and potato, milk and raw egg and sour milk and bread.

COMMENT

It has long been claimed by certain food faddists that in prescribing for the sick the essential point is to secure right combinations of food and to avoid wrong ones. It sounds reasonable, but we have not been able to find much convincing scientific evidence which would favor the theory or help the inquirer in the search for good combinations. The experiments here reported now show that the addition of certain foods to others does help or hinder their digestion, and the way is opened for the study of what may prove to be an important branch of dietetics. It is remarkable to see how the residues from two foods eaten together may be smaller than those of either food eaten separately, and it is

11. Alvarez, W. C., and Freedlander, B. L.: Rate of Progress of Food Residues Through the Bowel, J. A. M. A. **83**:576 (Aug. 23) 1924.

12. Plant, O. H., and Miller, G. H.: Effects of Morphine and Some Other Opium Alkaloids on the Muscular Activity of the Alimentary Canal. I. Action of the Small Intestine in Unanesthetized Dogs and Man, J. Pharmacol. & Exper. Therap. **27**:361 (June) 1926.

even more surprising to see that two almost completely indigestible substances such as raw egg and raw starch are well handled when given together.

Food eaten on the day following the ingestion of certain substances will be imperfectly digested. Alvarez has noted for years that persons just recovering from an attack of diarrhea often have so little ability to digest anything that for a few days they must partake sparingly of only the more digestible foods. When such a person begins immediately to eat everything, he sometimes suffers for months with indigestion, because the irritating residues from one poorly absorbed meal make it impossible for the subsequent meals to be digested properly.

The fact that purgation interferes with digestion and absorption must also be kept in mind when the physician is trying to help patients complaining of flatulence, abdominal discomfort and undernutrition. He cannot tell how much of the disturbance is due to purgation and how much to organic disease until he frees the bowel from insult not only by chemical irritants but also by the now commonly used mechanical irritants such as bran. Often great relief from symptoms can be obtained if the lower bowel is emptied, when necessary, by enemas of warm physiologic solution of sodium chloride.

These experiments suggest that certain patients may be injured if their food, during the process of preparation in the kitchen, is too finely chopped. Our observations also offer some explanation for the occasional deleterious effects of eating between meals. They also suggest that lactose should be given with care, and that its effects should be watched because it can do much harm.

SUMMARY

The amount of residue obtained after the digestion of various foods has been studied in six dogs, all deprived of the colon. The foods best digested were meat, rice, dextrose and fat in small amounts. These foods might well be used in the treatment of patients with diarrhea, and in the preoperative or postoperative care of patients with serious diseases of the colon, rectum or anus.

The combining of certain foods such as bread and milk and raw egg and milk, improved the digestion of both substances. Lactose, when given in considerable amounts, proved to be a laxative which interfered not only with the digestion of food eaten the same day but also with the digestion of food eaten the following day. Cheese and lard given one day also interfered with the digestion of food eaten the following day.

Meat was better digested when given in lumps than when given finely comminuted. The same was true of Swiss cheese. Raw meat was better digested than cooked meat.

Except in the case of a few foods, it was difficult to give enough so that the efficiency of digestion would fail. The dog usually protected himself by vomiting part of the meal.

Raw egg when not mixed with other foods was highly indigestible. Raw cornstarch given alone was almost completely indigestible.

The addition of lemon juice to food hurried the progress of residue through the bowel and interfered somewhat with the efficiency of digestion. Orange juice had little effect on digestion.

Pie crust did not seem to be much more indigestible than white bread. It was more digestible than graham bread.

Anxiety and excitement influenced adversely the efficiency of digestion.

Food when taken in one large amount was better utilized than when taken in several fractions at half-hourly intervals. Apparently each feeding stimulated peristalsis and caused the food to pass too rapidly through the bowel. Purgatives, sodium bicarbonate and barium sulphate interfered with digestion. Morphine slowed the progress of material through the bowel. It had little effect on the digestion of ground cooked meat, given at the same time, but it interfered with the digestion of meat given the next day.

ADDISON'S DISEASE IN A NEGRO

REPORT OF A CASE *

ANGELO M. SALA, M.D.
AND
MENDEL JACOBI, M.D.
NEW YORK

Addison's disease, contrary to the opinion of Addison himself, who felt that its recognition would show it of rather frequent occurrence, is relatively infrequent. Among white people in whom pigmentary changes lead to a diagnosis or suspicion of the disease with some ease, estimations of the frequency vary. Osler [1] stated that he saw only seventeen cases in the United States in twenty-one years. In 1925, Rowntree [2] was able to collect forty-seven cases (16 per hundred thousand cases registered) from the Mayo Clinic records of the preceding thirteen years.

Because of the difficulties of recognizing the disease in Negroes attendant on their color, the diagnosis of Addison's disease is apparently rarely suspected and still more infrequently made and confirmed. In a careful search of the literature for the last forty years, we have been able to find but four recorded cases, only two of which came to autopsy. We record a case occurring at the Harlem Hospital, where the greater portion of the patients are colored.

CASE REPORT

D. R., a colored man, aged 51, was admitted to the Harlem Hospital on July 10, 1927. No definite or detailed history was obtainable, except that he had been ill for about two months, rapidly losing weight and becoming very tired and weak. No pulmonary symptoms were present. He had been totally bedridden for the last two weeks; he had begun then to experience great difficulty in speaking, was exceedingly hoarse, and had some pain in his throat.

Physical examination revealed an acutely ill colored man, about 50 years of age. He was markedly emaciated. The respiration was shallow but equal on both sides; the breath sounds and vocal fremitus were diminished throughout; the percussion note was somewhat dulled generally with areas of high-pitched resonance scattered throughout the chest, and occasional moist râles were present. The heart sounds

* Submitted for publication, Dec. 10, 1929.

* From the Pathologic Laboratories, Harlem Hospital, and Brownsville and East New York Hospital, Brooklyn.

1. Osler, quoted by Dock and Lisser, in Osler, William, and McCrae, Thomas: Modern Medicine, ed. 3, Philadelphia, Lea & Febiger, 1926, vol. 5, p. 273.

2. Rowntree, L. G.: Studies in Addison's Disease, J. A. M. A. **84**:327 (Jan. 31) 1925.

were of poor quality, regular but distant; no murmurs were heard. The abdomen was scaphoid, the wall thin and emaciated; there were no palpable masses. The temperature on admission was 100 F.; the pulse rate, 110, and the respiratory rate, 40. Attempts at making blood pressure readings were unsuccessful. Stimulative treatment was immediately instituted, but there was no response, and the patient died twelve hours after admission. The antemortem diagnosis was probable malignancy with cachexia.

Autopsy was performed sixteen hours after death. The body was that of a poorly developed, markedly emaciated colored man, with loose skin that was boggy over the chest wall. The fingers were markedly clubbed. The abdomen was scaphoid. There was a large, oval, cystic bulge present in Scarpa's triangle on the right side. No pigmentation of the mucous membranes of the mouth or lips, or of the nail-beds was apparent. The larynx showed a dry, pale, smooth mucosa, without ulceration; no tubercles were present. In the right lobe of the thyroid there was a cystic adenoma. Above the pulmonary apex on the right, at the root of the neck, a firm, movable, deeply blackened anthracotic lymph node was present.

The interlobular fissures of both lungs were obliterated by firm, fibrous adhesions. The right and left upper lobes were firmly adherent to the lateral and posterior walls of the chest, and the pleural cavities were obliterated; the visceral pleura covering the lower lobes was dulled but not covered by exudate. In the right upper lobe, extending anteriorly from the extreme apex to the third rib there was a large, multiloculated cavity lined by a thin, shaggy, green, fibrinopurulent membrane. The loculi communicated with one another and with the eparterial bronchus. They were filled with thick, odorless, creamy material. The cavity was surrounded laterally and above by a thin strip of firm, airless, grayish-red lung tissue. Throughout the remainder of the right upper and lower lobes were numerous discrete and confluent, firm, grayish-white areas, sharply demarcated; in the right middle and left upper lobes such areas were rather more infrequent, while very few were present in the left lower lobe. The intervening lung tissue was crepitant. At the hilus, more particularly on the right side, were several discrete, large, firm anthracotic lymph nodes.

The heart weighed 200 Gm. The pericardium was thick and opaque. Little epicardial fat was present; the heart muscle (particularly the papillary muscle) was pale and diffusely permeated with yellow streaks. The coronary arteries, the aorta and the abdominal branches of the latter contained numerous subintimal, atheromatous and calcific plaques.

In the thickened omentum and the parietal peritoneum were numerous discrete, round, sharply demarcated nodules (about 3 mm. in diameter), firm and gritty, which cut with difficulty. A few old fibrous strands were present between the ileal and the jejunal coils. The liver weighed 1,200 Gm.; its surface was smooth and its lobular structure quite distinct on section. The spleen weighed 120 Gm.; its capsule was wrinkled and thickened; the corpuscles were distinct on the cut surface, and there were no tubercles. The kidneys weighed 235 Gm. each; bilateral double ureters were present, the two ureters opening into the same major calix on the right, into separate calices on the left, and into the bladder by four distinct openings. No other gross changes were present in the kidneys or bladder, nor in the gallbladder, bile ducts or pancreas.

The right suprarenal gland weighed 9 Gm.; it showed no gross morphologic changes. The left weighed 23.4 Gm. It covered the entire upper pole of the kidney, extending mesially caudad to overlap the renal hilus. It was roughly shaped like a bean; its capsule was everywhere intact, and it was easily separable from the kidney and the surrounding tissue. The entire gland was firm, almost

hard in consistency. On section, no cortex or medulla was apparent. Instead, several large, homogeneous, firm, irregularly outlined nodules of structureless, yellow, opaque tissue filled the entire organ, the largest measuring 3 by 1 cm. and occupying the entire upper half. Between these nodules were narrow, pale, dull brown strands of tissue resembling medullary tissue. No softened areas were present.

The right testis and epididymis were not grossly altered. No testis could be found on the left side either in the scrotum or in the widened inguinal canal or intra-abdominally. The left spermatic cord was a firm, grossly structureless mass of fibrous tissue.

The dorsal part of the spine was scoliotic to the left; the fifth, sixth and seventh vertebrae were wedge-shaped and collapsed anteriorly. The anterior and right lateral surfaces of the sacrum were roughened and eroded. Large, soft, caseous lymph glands covered its surface. In the right psoas muscle, from the level of the second lumbar vertebra downward into Scarpa's triangle, there was a large multiloculated cavity lined by a thick, shaggy, greenish necrotic membrane and filled with about a pint of odorless, greenish white pus. This cavity communicated with a smaller similar one lying in the tissues adjacent to the terminal sacrum and coccyx. The lumbar vertebral bodies or processes were not involved. The mesenteric lymph nodes showed no gross changes.

A smear from the cavity in the psoas showed acid-fast bacilli and gram-negative short bacilli, much fibrin, many lymphocytes and degenerated polymorphonuclear leukocytes.

Microscopically, the lungs (through the abscess wall) showed confluent caseous areas containing and surrounded by multinucleated giant cells. The adjacent alveolar walls were hyalinized and had no demonstrable capillaries; a few alveoli were filled with granular fibrin, varying numbers of lymphocytes and a few large endothelial cells, usually uninucleated, but grouped together and with indistinct cell borders. Sections through other portions of the lungs showed numerous minute tubercles composed of endothelial cells and central multinucleated giant cells in the alveolar walls, and often filling the alveolus. No septal or vascular fibrosis was apparent.

The right suprarenal gland showed only a paucity of fat vacuoles in the cortex; the left (region of the large mass in the upper half) showed a wide capsular zone of coarse, dense connective tissue diffusely infiltrated with lymphocytes. Immediately beneath this layer were a few small columnar cells containing a small amount of clear, homogeneous acidophilic cytoplasm and a small, deeply basophilic nucleus. These cells were arranged in coiled masses or short fascicular strands between a fine fibrous network. The remainder of the tissue consisted of caseous matter containing a few multinucleated giant cells. Strands of the subcapsular cells, poorly stained and often without nuclei, were present between the caseous masses. Sections through the lower portion of the suprarenal showed numerous tubercles, with caseous centers, lying in the cortex and medulla, the cells of both of which were compressed.

The liver showed marked fatty vacuolation of the cord cells. Here and there an occasional multinucleated giant cell surrounded by a few lymphocytes and necrotic liver cells was found in the parenchyma. No relation between these areas and the portal space or central vein was apparent.

The spleen showed numerous small necrotic areas irregularly scattered within the pulp. These areas showed no giant cells; in places, a few faintly outlined, elongated cells resembling epithelioid cells were seen.

Sections of the peritoneal nodules showed them to consist of calcific, granular material surrounded by fibrous tissue heavily infiltrated with lymphocytes. Sections of the abdominal lymph nodes showed no changes; those along the sacrum showed numerous caseous areas surrounded by multinucleated giant cells. Sections through the psoas abscess wall showed tuberculous granulation tissue. No tubercle formation or caseation was apparent.

COMMENT

The case reported is obviously one of long-standing pulmonary and bony tuberculosis, terminating rapidly after involvement of the non-cavitated pulmonary lobes and the suprarenal in a manner suggestive of blood stream invasion. The presence of the calcific nodules in the peritoneum is suggestive of an earlier, healed, peritoneal tuberculosis; their discrete arrangement even suggests a miliary distribution.

Of importance is the rapid course of the disease in its acute suprarenal involvement and the total absence of increased pigmentation.[3] In the other cases recorded in Negroes, pigmentary changes, especially darkening of the palms and soles, was a striking feature. None, however, was of such short clinical duration as our case. Of the two cases that went to autopsy, one showed cavitation of the suprarenal gland, the other extensive hyalinization and fibrosis, both indicative of a rather long-standing process. Of the recorded cases in Negroes, Lemann's[4] patient noted a darkening and discoloration of his forearms and face progressing over his entire body one year before death occurred. Lemann described his patient as of "stove-polish" or "shoe-black" color, especially the hands, face, forearms, feet and lower part of the legs, saying that "he was the blackest Negro seen in the clinic." Biopsy of the skin showed marked pigmentation of the chromatophores.

Two of Evans'[5] patients showed an increase of body pigmentation for more than one year; the third noted brown discolorations on the dorsa of her hands and feet for a year, and showed a slight increase in pigmentation over the borders of the tongue at the junction of the anterior and middle thirds. Of the two patients examined post mortem, Evans' showed scattered areas of black and dark brown pigmentation on the buccal mucosa, the hard and soft palate, and under the tongue, while Lemann's patient showed dark discolorations of the gums and dark spots on the tongue and buccal mucosa.

Our case presented symptoms for but two months. In the two cases just quoted, one patient[4] died one and one-half years after the onset

3. Chvostek, quoted by Hektoen: American Text-Book of Pathology, Philadelphia, W. B. Saunders Company, 1902, p. 923.

4. Lemann, I. I.: Addison's Disease: Report of Three Cases, Including One in a Negro, New Orleans M. & S. J. **78**:814 (June) 1926.

5. Evans, L. S.: Addison's Disease in the Negro: Report of Three Cases, Am. J. M. Sc. **176**:499 (Oct.) 1928.

of symptoms, the other,[5] five years after coming under observation and two years after the diagnosis was made. The other two patients whose cases were reported [5] were alive and in fair condition one year after the onset of the symptoms. The consensus of most authors is that an acute course is rare in white patients.[6]

Weakness as the presenting symptom was present in all cases. Gastro-intestinal symptoms occurred in four patients; vomiting was present in two,[5] usually without relation to meals and most often in the morning; diarrhea occurred in all four.[7] In all reported cases, as well as in our own, marked anorexia appeared early in the symptomatology.

Of considerable prominence in this group of cases are symptoms referable to the respiratory system and larynx. Our patient complained of soreness when talking; he was quite hoarse when seen in the hospital. One of Evans' patients had been so hoarse that she "could not talk above a whisper" for a month; no pain on talking is noted. Choking sensations were periodically present, accompanied by dizziness and a feeling of faintness in the case that came to autopsy. No note is made of any lesions of the larynx or respiratory tract other than the presence of two small tubercles in the right lung and an adherent pleura bilaterally. Our case likewise showed no lesions of the larynx; the pulmonary pathologic process does not appear to be sufficient to explain the pain and hoarseness. We feel that these symptoms are probably the expression of the general asthenia; the soreness noted was perhaps the result of repeated forcible attempts to speak loudly, with a consequent local laryngeal irritation.

In three of the cases recorded there was a past history suggesting tuberculosis: one of Evans' [5] patients had an abscess of the chest wall which was opened three years before the onset of pigmentary changes; another had a fistula in ano for several years, and a third had pneumonia associated with a pleurisy for one month ten years before observation. In the other case [4] there was a history of syphilis and gonorrhea. There was no history of familial tuberculosis present in any case.

Among white people, the disease is most frequent in males (Kaufmann, Timme [6]). In the short series of the disease in Negroes, three cases occurred in female and two in male patients. Whereas most of the cases in white persons occur between the twentieth and fortieth years, none of the Negroes was under 30; one was 36, and two were 51.

6. Osler (footnote 1). Kaufmann: Spezielle pathologischen Anatomie, ed. 8, Berlin & Leipzig, Walter de Gruyter & Company, 1922, vol. 11, p. 1004. Timme, W., in Cecil: Text-Book of Medicine, Philadelphia, W. B. Saunders Company, 1927, p. 1133.

7. Lemann (footnote 4). Evans (footnote 5).

Two of the previously recorded cases came to autopsy. Lemann's case showed in the suprarenal glands irregularly lobulated masses 8 by 2 cm., pale yellow and surrounded by fat. The cut surface of each was studded with numerous yellow areas of various size, some of the larger areas showing central necrosis, cavitation and calcification. Microscopically, these areas were typical tubercles. The lungs contained numerous tubercles of various sizes and a small cavity at the right apex. A few recent tubercles were present in the kidneys and retroperitoneal lymph glands. The case was apparently one of tuberculosis of the lungs and suprarenal gland of some duration, ending in a terminal miliary dissemination. Syphilitic aortitis was an associated condition.

In Evans' case, the left suprarenal was small, flat, firm and more nodular than the right. It was peculiarly hard on section; no cortex or medulla was distinguishable, the gross observation of a general fibrosis being made; no caseation was grossly visible. Histologically, the involved suprarenal gland showed extensive hyalinization and fibrosis and typical tubercles without necrosis. The lungs showed extensive pleural adhesions, slight anthracosis and several apical tubercles. The heart was atrophic (98 Gm.). The kidneys were small, flabby and atrophic, with flattened pyramids; there was a small tubercle on the surface of the right kidney. Microscopically, there were patches of cellular infiltration of the cortex near the capsule, with atrophic glomeruli, thickened glomerular capsule and shrunken tubules.

CONCLUSION

Including our own, only five cases of tuberculous involvement of the suprarenal glands in Negroes are on record, a rather surprising fact in view of the frequency of pulmonary and miliary tuberculosis in the race. In this series of cases, females were more frequently affected than males (3:2), as against an average of (2:1) greater frequency in males among white people. The age incidence in these cases was somewhat higher than in white people. Pigmentary changes did occur in the cases of considerable duration. These involved areas usually uninvolved in white people (nail-beds, soles and palms), although this distribution may be due merely to the more ready recognition of pigmentary changes in these areas in colored patients. Changes in general pigmentation may become extreme and extensive. In the acute forms, pigmentary changes may be absent.

The course in the cases recorded was more rapid than in white people. Nervous symptoms were not prominent in any case. Laryngeal symptoms—hoarseness, pain on speaking and a choking sensation—were prominent, or even presenting, symptoms in this group of cases. No local evidence to explain these symptoms was present. Asthenia was

a prominent symptom; anorexia was marked. In all cases, except in those in which pigmentary changes and gastro-intestinal and asthenic symptoms were marked, the diagnosis, always difficult to make in white people, was extremely difficult; in the acute case, it was not often made except at autopsy.

SUMMARY

The fifth case of Addison's disease in a Negro, with autopsy observations, is recorded in detail. The other recorded cases are reviewed.

A comparison is made with the disease in white persons. The difficulty of diagnosis and the prominence of symptoms not usually stressed in white people are emphasized. The laryngeal and respiratory symptoms are stressed particularly.

CHEMISTRY AND METABOLISM IN EXPERIMENTAL YELLOW FEVER IN MACACUS RHESUS MONKEYS

II. NITROGEN METABOLISM *

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In the preceding paper,[1] determinations of nonprotein nitrogen and its individual nitrogenous components in the blood of normal *Macacus rhesus* monkeys and similar animals infected with yellow fever were presented. It was shown that in the terminal stage of yellow fever, the amino-acid nitrogen in the blood increased greatly, both in absolute magnitude and in proportion to total nonprotein nitrogen and other individual nitrogenous constituents. Urea nitrogen, on the other hand, did not rise, or rose little in proportion to amino-acid and total nonprotein nitrogen. In a few instances it actually diminished. The nature of the observed changes indicates that the chief disturbance of nitrogenous metabolism in yellow fever is the destruction or impairment of the power of the liver to deaminize amino-acids and to form urea.

However, definite conclusions cannot be drawn from studies of the blood alone. In order to estimate the rates of formation and destruction of nitrogenous metabolites, simultaneous studies of blood nitrogen and total nitrogen metabolism were carried out on a series of monkeys infected with yellow fever. These studies, which are reported in this paper, confirm the results of studies on the blood reported in the preceding article.[1] Uric acid metabolism remains essentially unaltered, but the functions of deaminization and urea formation are profoundly injured.

The technic employed in the metabolism experiments was described in detail in the first paper.[1]

Only protocols of typical experiments are given. Those which have been chosen are, however, representative and afford a complete picture of the metabolic disturbances encountered.

* Submitted for publication, Jan. 30, 1930.

1. Wakeman, A. M., and Morrell, Clare, A.: Chemistry and Metabolism in Experimental Yellow Fever in Macacus Rhesus Monkeys: I. The Concentration of Nonprotein Nitrogenous Constituents in the Blood, Arch. Int. Med. **46**:290 (Aug.) 1930.

Protocols of the experiments are given in the following paragraphs, and corresponding metabolism data are presented in tables 1 to 5 inclusive.

CHANGES IN THE NONPROTEIN NITROGENOUS CONSTITUENTS OF BLOOD AND URINE DURING THE TERMINAL STAGE OF YELLOW FEVER: EXPERIMENTAL WORK

EXPERIMENT 1.—*Macacus rhesus* M3, a large male in good condition, was fed diet no. 3, beginning on April 28. On April 29, the animal was infected by a mosquito bite. On May 2, it was quite normal in appearance, and a blood sample

TABLE 1.—*Changes in the Nonprotein Nitrogen Constituents of Blood and Urine During the Terminal Stage of Yellow Fever in Monkey M3*

Blood Taken	Non-protein Nitrogen, Mg. per Cent	Urea Nitrogen, Mg. per Cent	Amino-Acid Nitrogen, Mg. per Cent	As per Cent of Total Nitrogen: Urea Nitrogen	As per Cent of Total Nitrogen: Amino-Acid Nitrogen	Sugar Mg. per Cent	Comment
May 2, 2 p. m., from right foreleg	43	20	8	46	18	109	Last meal at 9:30 a. m.
May 3, 8:10 a. m., from right foreleg	61	17	15	28	25	66	Last meal at 4:30 p. m. May 2
May 3, 4:10 p. m., from right foreleg	67	15	17	22	25	113	Last meal at 9:10 a. m.
May 3, 10:15 p. m., from heart; 10 minutes post-mortem	99	5	33	5	33	15	Last meal at 5 p.m.

Date	Urine Volume, Cc.	Urine Nitrogen as: Total, Mg.	Urea, Mg.	NH_3, Mg.	Urea + NH_3, Mg.	Amino-Acid, Mg.	Rest, Mg.	As per Cent of Total Nitrogen: Urea + NH_3	Amino-Acid	Acetone	Temperature: A. M.	P. M.
April 28	37	485	563	47	610	17	358	63	2	Negative	102.0	102.7
April 30	35	445	235	25	260	10	175	58	2	Negative	102.0	102.7
May 1	65	712	407	45	452	18	242	63	3	Negative	102.7	103.5
May 2 *	65	836	504	36	540	23	273	65	3	Negative	104.4	104.9
May 2 *	65	836	504	36	540	23	273	65	3	Negative	104.4	104.9

* Weight = 4.3 Kg.
† Died at 10:05 p. m.
‡ Specimen voided at 3:00 p. m.

was taken. On May 3, it was drowsy but vigorously resisted handling when the second blood sample was taken at 8:10 a. m. It was lying down, weak and listless at 4:10 p. m. when the third blood sample was taken; at 7:10 p. m. it was lying down and was unconscious. Death occurred at 10:05 p. m. A sample of blood was taken from the heart ten minutes post mortem.

The urine had been tested each day for acetone with the possibility in mind that there would be a long premortal period with a low blood sugar level. Such was not the case, and it was not until shortly before death that the blood sugar fell below the normal level.

The results of blood and urine studies are recorded in table 1.

This experiment was chosen for presentation because it illustrates the rapidity with which the blood changes develop during the last hours of the disease. It does not permit exact estimation of nitrogen metab-

olism, because dietary and stool nitrogen were not determined. The most striking feature of the experiment is the absolute diminution of blood urea in the last six hours of life after urine formation had ceased.

Studies were made to investigate more thoroughly the changes occurring in nitrogen metabolism during the terminal period of yellow fever. Protocols of four typical experiments are given here. One of the animals (M 1) recovered, and three (M 5, M 4 and M 2) died.

EXPERIMENT 2.—*Macacus rhesus* M1, a large male, in good condition, was fed diet no. 1 beginning on August 14; metabolism studies were begun on August 18. On September 1, the monkey was inoculated with 0.5 cc. of Asibi strain blood virus; on September 7, it was reinoculated with 1 cc. of the same virus. September 10, the animal appeared ill, and was found lying down, but arose if disturbed; it vomited 60 cc. of substance between 5 and 8 p. m.

On September 11, the animal was weak after the withdrawal of 12 cc. of blood. The usual diet was replaced by 15 Gm. of cane sugar and 0.2 Gm. of salt in 60 cc. of water, given both morning and evening; at 5 p. m., the animal was distinctly weak. The morning of September 12 found it somewhat brighter, but it vomited in the afternoon. When offered 15 Gm. of cane sugar and 0.3 Gm. of salt in 60 cc. of solution at 5 p. m., the monkey drank a small quantity and upset the remainder. On September 13, it was decidedly better, and appeared quite well. On September 15, a small abscess was drained at the site of the incision over the femoral artery, which healed uneventfully. The monkey appeared normal for the remainder of the experiment.[1]

The blood used in the first inoculation was taken on the first day of fever from a monkey that had died on the seventh day after inoculation. Three other monkeys were inoculated with 1 cc. of this blood, two of them one day before, one of them one day after the inoculation of M1; all died. The inoculation on September 7 was made with blood of a monkey taken on the first day of fever. This monkey died after a short illness. Subsequent inoculation of M1 with blood from a monkey which later died, taken on the first day of fever, produced no reaction. From a consideration of this evidence, it seems reasonable to assume that the elevation in temperature produced in M1 by the injection of virulent blood represented an attack of yellow fever which rendered the animal subsequently immune.

The reaction of day and night urine to litmus was tested in each twelve hour specimen. In general, the night urine was more acid than the day. Most specimens of urine passed from 8 a. m. to 8 p. m. were slightly alkaline or neutral, while those passed from 8 p. m. to 8 a. m. were neutral or more often acid. From September 10 to 14 both were distinctly acid. The volume of the night specimen was usually greater than the day urine.

No glycosuria was observed from August 27 to September 16.

The foam test for bile was negative throughout the experiment, although from September 11 to 14 the urine was abnormally dark in color.

On September 11, 131 mg. of protein was present in the urine and a trace and a faint trace were present on September 12 and 13, respectively.

The van den Bergh test revealed no bile pigment in the serum except a faint trace in the blood taken on September 11.

The results of metabolism and blood studies are presented in table 2. The figures represent the average values from duplicate analyses. All decimals have been omitted in this and other tables. This explains the apparent constancy of the daily excretion of uric acid and amino-acid nitrogen in some periods. Differences of less than a milligram of nitrogen are insignificant for the purpose of this study, and convenience and clearness justify their omission in the tables. The figures representing the urine nitrogen fractions as percentage of total nitrogen have also been reduced to the nearest whole number, except in the case of uric acid.

The nitrogen balance was calculated by subtracting the urine plus the feces nitrogen from the diet nitrogen. The rest nitrogen is the remainder after subtraction has been made from total nitrogen of the determined nitrogen fractions.

There is little disturbance of blood nonprotein nitrogen metabolism in this, as in other cases in which recovery occurred. Failure to take the full diet, rise of urinary nitrogen and production of a negative balance from September 10 to 12, inclusive, are the only signs of disease evident in the nitrogen metabolism.

EXPERIMENT 3.—*Macacus rhesus* M5, a large female, in good condition, was fed diet no. 2, beginning on September 2; the metabolism studies were begun on September 13.

On September 22, the blood sample was taken and the monkey was inoculated with 1 cc. of Asibi strain blood virus. It vomited 8 cc. of the diet at 2 p. m. but showed no other signs of illness. On September 25, the monkey vomited 5 cc. of its diet between 2:30 and 4:30 p. m., but it looked well.

On September 26, the animal vomited a small amount of its food at 7 p. m., and on September 27, it was definitely less active; it vomited 5 cc. of the diet at 10:30 a. m. and 5 cc. at 8 p. m. On September 28, a blood sample was taken. The monkey was bright and active in the morning, but lay down in the afternoon. On September 29, it was weaker, lying down when undisturbed. At 11:30 p. m. it was very weak. Death occurred the following day at 6:15 a. m.

At 6:30 a. m., 25 cc. of blood was removed from the heart. When the body was removed from the cage at 6:30 a. m., about 60 cc. of black fluid poured from the nose and mouth. Analysis of this material showed it to contain 198 mg. of nitrogen and 650 mg. of chloride. Five cubic centimeters of dark-colored urine was taken from the bladder, which was then washed with distilled water.

In this experiment the day and night urine was analyzed separately, on the supposition that during an attack of yellow fever, alterations might be more easily demonstrated in the shorter periods. However, this was not found to be the case and only the results of twenty-four hour periods are presented in table 3. The small day volume compared with the night volume was attended by the excretion of larger amounts of nitrogen during the night.

The nonprotein nitrogen in the serum on September 28 was found to be 28.6 mg. per cent of the serum. Since this value was within the

TABLE 2.—*Nitrogen Metabolism During Yellow Fever in Monkey M1*

Date	Weight, Kg.	Urine Volume, Cc.	Total Nitrogen: Diet, Mg.	Urine, Mg.	Stool, Mg.	Balance, Mg.	Urine Nitrogen as: Urea, Mg.	NH3, Mg.	Urea + NH3, Mg.	Amino-Acid, Mg.	Creatinine, Mg.	Uric Acid, Mg.	Rest, Mg.	Organic Acid, Cc. Tenth Normal	Phosphorus, Mg.	Serum Nonprotein Nitrogen, Mg. per Cent	Temperature, F.: A. M.	P. M.
August 28	2.88	38	810	454	110	246	385	16	401	6	35	1	11	52	10	66	.....	.....
August 29		45	810	583	110	117	416	21	437	8	39	2	97	55	9	..	.....	.....
August 30		48	810	676	110	124	432	23	455	7	39	2	73	51	11	..	.....	.....
August 31		48	810	709	110	—9	477	28	505	6	38	2	158	51	18	..	.....	.....
Average		45	810	581	110	119	428	22	449	7	38	2	85	52	12			
Per cent of total nitrogen		..	...	...	...	...	73	4	77	1	6	0.3	14					
September 1*	2.88	33	810	275	70	465	146	23	170	4	27	1	73	28	12	56	.....	103.2
September 2		..	810	419	70	321	286	27	313	10	40	2	54	50	5	..	101.2	102.2
September 3		52	810	525	70	215	409	28	437	10	39	2	37	58	6	..	102.7	102.4
September 4		43	810	543	70	199	404	26	430	8	38	2	65	50	13	..	100.9	102.9
September 5		50	810	667	70	73	490	25	515	10	43	2	97	60	9	..	101.9	104.1
Average		48	810	538	70	2 2	397	26	424	9	40	2	63	54	8			
Per cent of total nitrogen		..	...	...	...	...	74	5	79	2	7	0.4	12					
September 6	2.96	42	805	635	43	127	492	26	518	9	44	2	62	55	14	36	102.1	102.8
September 7		41	820	645	43	132	383	17	400	8	45	2	190	62	28	..	103.2	102.9
September 8		41	835	593	43	199	378	20	398	8	43	2	142	50	15	..	103.1	103.4
September 9		51	850	737	43	70	419	21	440	9	47	2	239	54	11	..	102.7	103.6
Average		44	827	665	43	132	418	21	439	9	45	2	158	55	17			
Per cent of total nitrogen		..	...	...	...	...	63	3	66	1	7	0.3	24					
September 10		—†	430	1,041	43	—654	575	30	605	21	45	4	366	..	..	..	104.2	105.1
September 11	2.95	71	...	1,018 007	.. 43	—1,040	624	103	727	20	54	6	190	95	25	44	104.6	106.3
September 12		—†	430	873	67	—486	412	82	494	11	48	3	317	..	81	..	102.8	102.5
September 13		34	829	530	67	232	279	58	337	9	41	3	140	52	12	..	100.9	103.7
Average		52	563	800	55	—487	472	68	540	15	47	4	253	73	39			
Per cent of total nitrogen		..	...	...	...	...	55	8	63	2	5	0.4	29					
September 14		29	793	495	67	231	274	33	307	8	39	3	138	49	21	..	102.8	102.6
September 15*		34	793	335	67	391	...	..	...	..	28	..	...	..	..	..	101.4	101.4
September 16	2.84	56	793	403	67	324	231	27	258	10	38	2	95	37	6	..	101.9	102.3
September 17		..	...	...	...	...	...	..	...	..	..	..	...	..	..	40	.....	.....
Average		42	793	449	67	277	252	30	282	9	38	2	116	43	14			
Per cent of total nitrogen		..	...	...	...	...	56	7	63	2	8	0.4	26					

* Some urine lost. Figures not included in average.
† Urine contaminated with vomit. Volume not measured.

normal range, normal values for the nitrogenous constituents of whole blood were assumed. Blood taken from the heart fifteen minutes post mortem contained 65.8 mg. per cent of nonprotein nitrogen; 18.2 mg. per cent of urea nitrogen; 14.5 mg. per cent of amino-acid nitrogen; 0.9 mg. per cent of uric acid, 2.3 mg. per cent of creatinine and only a trace of ammonia nitrogen.

The metabolism data from this experiment are given in table 3.

EXPERIMENT 4.—*Macacus rhesus* M4, a medium-sized male, in rather poor condition, with thin hair and puffy eyelids, feebly resisted handling and was seen to be lying down occasionally. Diet no. 3 was begun on October 22 and the metabolism studies on October 27. On October 31 and November 1, an unknown amount of urine was lost. On November 2, an inoculation with 0.5 cc. of Asibi strain blood virus was given. On November 5, the monkey was lying down and was unconscious; a slight diarrhea was observed. The animal died at 11:15 p. m.

Blood was obtained from the heart ten minutes post mortem. About 8 cc. of urine was recovered from the bladder. Autopsy revealed a well advanced case of yellow fever. No other abnormalities were discovered to explain why the monkey had been in such poor general condition.

This monkey presented a consistently negative nitrogen balance, which increased during the febrile period. The slight diarrhea observed on the last day of life greatly increased the stool nitrogen for this period.

The results of metabolism and blood studies are presented in table 4.

EXPERIMENT 5.—*Macacus rhesus* M2, a large male, was in excellent condition. It was fed diet no. 3, beginning November 8, and the metabolism studies were begun November 15.

On November 19, an inoculation with 1 cc. Asibi strain blood virus was given. The animal vomited 5 cc. of the diet at 8:30 a. m., but showed no other signs of illness. On November 22, it appeared weak and did not resist handling as it usually had. At 4 p. m. it vomited 15 cc. of the diet. On November 23 it vomited and again on November 24, when no feces were passed. On November 25 the monkey was much weaker; no feces were passed. On November 26, weakness increased, and by evening the animal was prostrated. No feces were passed on this day. On November 27, there were large diarrheal stools at 8 a. m. The monkey was unconscious at this time, and died at 10:15 a. m.

The results of metabolism and blood studies are presented in table 5. That stool nitrogen is low in period 2 and high in period 3 is probably referable to the constipation and diarrhea, respectively, which occurred in these periods. The urine on November 22 was light green. On November 26, 312 mg. of protein was found in the urine. There was a striking diuresis during the last day of life that persisted until six hours before death. At 2 a. m. on November 27, 30 cc. of urine was passed, and at 4 a. m., 29 cc. At autopsy, about 0.5 cc. was found in the bladder, and about 150 cc. of black fluid was recovered from the stomach. Since this was greater in volume than the food given on the preceding day, it has been assumed that no food was absorbed on November 26.

The results of the experiment are given in table 5.

TABLE 3.—*Nitrogen Metabolism During Yellow Fever in Monkey M5*

Date	Weight, Kg.	Urine Volume, Cc.	Total Nitrogen: Diet, Mg.	Urine, Mg.	Stool, Mg.	Balance, Mg.	Urine Nitrogen as: Urea, Mg.	NH_3, Mg.	Urea + NH_3, Mg.	Amino-Acid, Mg.	Creatinine, Mg.	Uric Acid, Mg.	Rest, Mg.	Organic Acid, Cc. Tenth Normal	Phosphorus, Mg.	Serum Nonprotein Nitrogen, Mg. per Cent	Temperature, F.: A. M.	P. M.
September 18	2.43	41	467	207	60	200	105	22	127	7	25	1	47	..	..	38	.....	103.6
September 19		51	467	275	60	132	125	26	151	12	27	1	84	..	..	..	102.5	102.6
September 20		54	467	233	60	174	117	21	138	7	26	1	61	58	2	..	101.7	102.8
September 21*		45	467	204	60	203	106	19	125	..	25	..	..	51	2	..	101.9	103.1
Average		48	467	238	60	169	116	23	139	9	26	1	64	54	2			
Per cent of total nitrogen		..	...	...	...	...	49	10	59	4	11	0.4	27					
September 22*	2.52	..	467	...	26	...	...	..	...	..	..	..	...	..	..	30	101.6	102.4
September 23		26	467	145	26	296	54	19	73	4	26	1	41	..	..	..	102.0	103.0
September 24		49	447	346	26	75	208	21	229	9	23	1	82	..	2	..	102.5	103.7
September 25		66	387	478	26	−117	316	14	330	6	30	1	111	..	..	..	102.4	104.0
September 26*		49	447	364	26	57	...	23	...	7	29	1	...	..	..	..	104.1	104.6
September 27		..	387	461	26	−100	267	40	307	10	31	2	111	..	..	..	103.2	103.6
Average		47	417	357	26	77	211	23	234	1	27	1	86					
Per cent of total nitrogen		..	...	...	...	...	59	6	65	2	7	0.3	24					
September 28	2.47	60	429	372	53	4	187	79	266	13	33	4	62	..	..	29	101.9	104.9
September 29 †		53	223	237	53	−67	116	54	170	5	21	4	37	110	64	..	103.7	106.2
Average		56	326	304	53	−31	151	66	217	9	27	4	49					
Per cent of total nitrogen		..	...	...	...	...	50	22	72	3	9	1	16					

* Figures not included in average.

† Died at 6:15 a. m., September 30.

TABLE 4.—*Nitrogen Metabolism in Yellow Fever in Monkey M4*

Date	Weight, Kg.	Urine Volume, Cc.	Total Nitrogen				Urine Nitrogen as							Organic Acid, Cc. Tenth Normal	Phosphorus, Mg.	Blood Nitrogen as			Temperature	
			Diet, Mg.	Urine, Mg.	Stool, Mg.	Balance, Mg.	Urea, Mg.	NH3, Mg.	Urea + NH3, Mg.	Amino-Acid, Mg.	Creatinine, Mg.	Uric Acid, Mg.	Rest, Mg.			Non-protein Nitrogen, Mg. per Cent	Urea, Mg. per Cent	Amino-Acid, Mg. per Cent	A. M.	P. M.
October 27	....	91	446	508	40	—102	...	73	...	..	26	..	..	72	27	..	..	..	102.1	103.0
October 28	....	77	446	481	40	— 25	...	27	...	..	28	..	..	61	26	..	..	..	102.2	102.6
October 29	....	81	446	432	40	— 26	...	38	...	..	28	..	..	50	28	..	..	..	102.2	102.7
Average	....	83	446	457	40	— 51	...	46	...	..	28	..	..	61	27					
Per cent of total nitrogen	....	..	...	...	..	...	...	10	...	..	6									
November 2	2.52	41	446	420	41	— 15	280	37	317	6	27	1	69	46	35	24	10	5	.....	103.4
November 3	....	43	424	431	41	— 48	285	47	332	6	27	2	64	47	..	..	..	..	102.4	102.2
November 4	....	57	297	615	119	—437	431	46	447	6	51	2	99	48	52	..	..	..	103.1	103.9
Average	....	47	380	488	67	—166	332	43	375	6	28	2	77	47	43					
Per cent of total nitrogen	....	..	...	...	..	...	68	9	77	1	6	0.4	16							
November 5*	....	38	220	346	119	—340	255	23	278	3	15	1	49	45	45	50	28	12	104.3	105.7
Per cent of total nitrogen	....	..	...	...	..	...	73	7	80	1	4	0.3	14							

* Died at 11:15 p. m.

TABLE 5.—*Nitrogen Metabolism During Yellow Fever in the Case of Monkey M2*

Date	Weight, Kg.	Urine Volume, Cc.	Total Nitrogen: Diet, Mg.	Total Nitrogen: Urine, Mg.	Total Nitrogen: Stool, Mg.	Total Nitrogen: Balance, Mg.	Urine Nitrogen as: Urea, Mg.	Urine Nitrogen as: NH_3, Mg.	Urine Nitrogen as: Urea + NH_3, Mg.	Urine Nitrogen as: Amino-Acid, Mg.	Urine Nitrogen as: Creatinine, Mg.	Urine Nitrogen as: Uric Acid, Mg.	Urine Nitrogen as: Rest, Mg.	Organic Acid, Cc. Tenth Normal	Phosphorus, Mg.	Blood Nitrogen as: Non-protein Nitrogen, Mg. per Cent	Blood Nitrogen as: Urea, Mg. per Cent	Blood Nitrogen as: Amino-Acid, Mg. per Cent	Temperature: A. M.	Temperature: P. M.
November 15.....	3.42	..	...	...	...	...	...	..	...	..	..	..	..	..	..	..	..	..	102.1	103.1
November 16.....	....	92	450	595	81	4	225	32	257	7	47	2	52	50	14	..	..	..	102.3	103.9
November 17.....	....	89	450	300	81	69	160	30	190	12	45	2	51	35	16	..	..	..	102.7	102.8
November 18	....	51	450	226	81	143	90	23	113	8	46	2	57	41	11	35	..	9	101.8	102.8
Average.....	....	77	450	297	81	72	158	28	186	9	46	2	53	42	14					
Per cent of total nitrogen.....	....	..	...	...	..	...	53	9	62	3	15	0.7	18							
November 19.....	....	97	430	305	17	108	182	27	209	6	46	2	42	36	11	..	..	..	102.6	102.8
November 20.....	....	58	450	247	18	185	122	24	146	5	46	2	48	35	10	..	..	..	102.1	103.3
November 21.....	....	84	450	370	17	63	217	20	237	6	58	2	67	50	2	..	..	..	102.1	103.6
November 22*....	....	22	385	308	18	59	186	9	195	6	45	2	60	23	7	..	..	..	104.1	104.1
November 23.....	....	51	445	672	17	—244	480	30	510	9	60	4	89	..	..	..	..	..	102.9	102.9
November 24.....	3.46	30	225	475	18	—268	285	43	328	6	50	4	87	..	45	29	13	8	101.7	102.3
Average.....	....	64	400	414	17.5	—31	257	29	286	6	52	3	66	34	17					
Per cent of total nitrogen.....	....	..	...	..	..	...	62	7	69	1	12	0.7	16							
November 25.....	....	44	445	630	138	—323	339	81	420	9	60	6	135	44	49	..	..	..	102.2	105.1
November 26.....	....	129	0	{450† {500	138	—588	185	93	278	12	59	9	92	189	97	..	..	..	104.9	106.6
November 27‡....	....	..	...	...	..	...	...	..	...	..	..	..	..	..	..	55¶	9	26	.....	.....
											..	..	..	..	..	67#	10	28	98.0	.....
Average.....	....	86	222	540	138	—450	262	87	349	10	59	7	113	116	73					
Per cent of total nitrogen.....	....	..	...	...	..	...	48	16	65	2	11	1	21							

* Urine lost. Figures not included in average.
† Nonprotein nitrogen.
‡ Died at 10:15 a. m.
¶ Blood taken at 8 a. m.
# Blood taken at 10.30 a. m.

AMINO-ACID INJECTION EXPERIMENTS

The ability of a hepatectomized dog to dispose of injected amino-acid has been investigated by Bollman, Mann and Magath.[2] They found in those animals in which the kidneys remained, a decrease in urea nitrogen of the blood and urine and large increases in amino-acid nitrogen. Only a fraction of the amino-acid nitrogen injected was excreted in seven or eight hours following the injection. They concluded that no deaminization occurred in animals after removal of the liver.

Yellow fever at post mortem presents a striking picture of liver degeneration. How soon and to what extent this degeneration occurs probably depends on the rapidity of the onset and the final outcome of the disease. As an attempt to supplement our previous work and to demonstrate as clearly as possible a loss of ability to deaminize amino-acids in yellow fever, the following experiments were carried out.

Two animals were given injections of glycin solution and the changes in blood and urine nitrogen studied. Glycin was used, since it was the only amino-acid available. Both of the animals used for this purpose developed a fever and showed some evidences of illness, but recovered. Each monkey received 250 mg. of glycin nitrogen intravenously on two separate occasions, once during the preliminary control period and again during the fever of yellow fever. Blood and urine were studied before and at frequent intervals after the injections. In neither case did the reactions during the febrile periods differ appreciably from those during the control periods.

Metabolism Study of a Monkey That Died of a Febrile Disease of Unknown Etiology.—As a basis of comparison with yellow fever, a nitrogen study of a monkey which died after a fever of unknown etiology (autopsy revealed some pneumonia) is included.

EXPERIMENT 6.—*Macacus rhesus* M6, a large female, apparently in good condition, was fed on diet no. 3, beginning on January 18, and the metabolism studies were begun on January 23. No feces were passed from January 21 until January 25, at which time a light yellow stool, about the size of a large kidney bean and of firm consistency, was passed; the monkey vomited 20 cc. of the diet and appeared ill and generally weak, showing rapid shallow respiration.

On January 26, the animal vomited; a slight diarrhea occurred. The condition was the same as on January 25. On January 28, there was violent diarrhea and the urine was grass green in color. On January 29, the diarrhea continued, and the monkey died suddenly without the signs of prostration that precede death from yellow fever.

The urine and blood analysis are recorded in table 6.

2. Bollman, J. L.; Mann, F. C., and Magath, T. B.: Am. J. Physiol. **78**:258, 1926.

TABLE 6.—*The Nitrogen Metabolism of Monkey M6 with a Fatal Illness of Unknown Origin*

Date	Urine Volume, Cc.	Urine Nitrogen as							As per Cent Total Nitrogen						Blood Nitrogen as			Temperature	
		Total, Mg.	Urea, Mg.	NH_3, Mg.	Urea + NH_3, Mg.	Amino-Acid, Mg.	Creatinine, Mg.	Rest, Mg.	Urea, Mg.	NH_3, Mg.	Urea + NH_3, Mg.	Amino-Acid, Mg.	Creatinine, Mg.	Rest, Mg.	Non-protein Nitrogen, Mg. per Cent	Urea, Mg. per Cent	Amino-Acid, Mg. per Cent	A. M.	P. M.
January 23.....	101	547	339	37	376	8	54	109	62	7	69	1	10	20	..	..	...	101.8	102.1
January 24.....	36	635	455	43	498	6	43	88	72	7	79	1	7	13	..	..	..	102.7	103.4
January 25.....	60	1,165	890	90	980	9	61	115	76	8	84	1	5	10	..	..	...	102.9	104.5
January 26.....	59	1,174	842	88	930	11	57	176	72	7	79	1	5	15	41	18	7	102.9	104.0
January 27.....	58	1,110	765	75	840	10	60	200	69	7	76	1	5	18	..	..	...	103.2	103.9
January 28*	..	.....	...	..	...	..	..	...	..	..	..	..	..	..	25	11	6	101.5	103.1
January 29†	..	.....	...	..	...	..	..	...	..	..	..	..	..	..	35‡	12	9	101.1	102.5

* Violent diarrhea. Urine contaminated.

† Died at 4:30 p. m.

‡ Blood taken five minutes post mortem.

COMMENT

The excretion of uric acid appears to increase slightly during the last few hours of life (tables 2 to 5, inclusive). However, these increases are small, corresponding in magnitude to those observed in the blood,[1] and do not indicate any grave injury to uricolytic function. The quantities excreted during the control periods are in agreement with those reported by Hunter and Givens.[3] Although in some experiments the uric acid output became from two to four times the normal, the total changes amounted to only a few milligrams at the most and are not of the same magnitude as those observed by Mann and his associates.[4] In hepatectomized dogs which they studied, uric acid appeared in the urine in such high concentration that it formed a heavy flocculent precipitate.

Attention has already [1] been called to species differences in the metabolism of uric acid. Although the power of uricolysis is not usually attributed to human liver, Quincke [5] found enormous quantities of uric acid in the urine of patients who had recovered from acute yellow atrophy of the liver. In Rabinowitch's [6] case, however, blood uric acid remained normal, and no appreciable quantities were found in the urine. According to Wells,[7] the liver of *Macacus rhesus* is the only organ of that animal which is capable of destroying uric acid in vitro. If this is true also of the living animal, and if, as Mann [8] has pointed out with regard to the dog, "the destruction of uric acid is the most easily injured known function of the liver with relation to metabolism," accumulation and excretion of uric acid in monkeys with yellow fever should be far greater than any observed. Either uricolysis in monkeys is not so easily disturbed by the hepatic lesions of yellow fever, or it is a function not confined exclusively to the liver.

Creatinine excretion was remarkably constant from day to day during control periods. A rise of temperature was always accompanied or preceded by an increase of urinary creatinine. The quantities excreted roughly paralleled the temperature, and the peak of the fever was usually accompanied by the maximum creatinine output. The minimal accumulations of creatinine in the blood,[2] in the face of increased creatinine excretion, indicate neither severe renal damage nor distur-

3. Hunter, A., and Givens, M. H.: J. Biol. Chem. **13**:371, 1912-1913; ibid. **17**:37, 1914.

4. Bollman, Mann and Magath (footnote 2).

5. Quincke in Nothnagel: Specielle Pathologie und Therapie, Vienna, vol. 18.

6. Rabinowitch, I. M.: J. Biol. Chem. **83**:333, 1929.

7. Wells, H. G.: J. Biol. Chem. **7**:171, 1910.

8. Mann, F. C.: Modifying Physiologic Processes Following Total Removal of the Liver, J. A. M. A. **85**:1472 (Nov. 7) 1925.

bance of creatinine production. Mann and his co-workers [2] found that the excretion of creatinine in dogs was not appreciably affected by hepatectomy.

In the control periods, ammonia nitrogen in the urine formed a rather high proportion of the total nitrogen excreted. Only in the case of the monkey M1 was it as low as 4 per cent of the total nitrogen. In the other monkeys it averaged between 7 and 10 per cent. During the febrile and terminal periods, the ammonia as a rule increased at the expense of urea nitrogen. In M 1, M 5 and M 2 (tables 2, 3 and 5) the ammonia output during the height of the fever became from three to four times the normal average. Monkey M 4 (table 4) had a higher output during the control period than during the yellow fever period. In the monkey M 5 (table 3), ammonia nitrogen formed 22 per cent of the total nitrogen during the last two days of life.

This increased output of ammonia may be ascribed to the larger output of organic acid and phosphorus during the illness. Bollman, Mann and Magath [2] found the ammonia excretion during the first six hours after dehepatization to be about three times greater than normal.

The amounts of organic acids and phosphorus in the urine did not parallel the total nitrogen excreted but were usually largest on the last day of life regardless of any decline in the nitrogen excretion. In monkeys M1, M5 and M2 (tables 2, 3 and 5), the organic acid and phosphorus excretions had increased during yellow fever from two to five times the normal amount, while in M4 (table 6) they were higher during the control period. Roman [9] considers that the increased ammonia excretion reported during acute yellow atrophy is probably due to the presence in the urine of organic acids, which were identified as sarcolactic, oxymendelic, acto-acetic and β-oxybutyric acids. The organic acids in yellow fever are presumed to be acid products of protein autolysis. There seems to be no relationship between the amounts of organic acid and rest nitrogen excreted.

Briefly stated, the ammonia nitrogen of the blood does not change noticeably during yellow fever, while the amount excreted in the urine does increase at the expense of urea nitrogen. This increase in urinary ammonia is coincident with larger excretions of organic acid and phosphorus. The organic acids and phosphorus in the urine increase during the febrile and terminal periods without apparent relationship to the amount of rest nitrogen.

Alterations in amounts and proportions of urea and amino-acid nitrogen fractions of blood during yellow fever present a picture suggestive of loss of the deaminizing and urea-forming functions of

9. Roman, B.: Acute Yellow Atrophy of the Liver, Arch. Path. **4**:399 (Sept.) 1927.

the liver. The quantities and proportions of the two fractions in the urine do not, at first sight, support such a conclusion. The excretion of urea, ammonia, amino-acids and rest nitrogen during yellow fever presents no striking features. Normally, in spite of the controlled nitrogen intake, the amount of nitrogen in the urine from day to day was far from constant. The amount of total nitrogen excreted invariably increased during fever indicating a toxic destruction of protein. *Macacus rhesus* M6 (table 6), during a fever of unknown origin, had the same large increase in nitrogen excretion as was displayed in yellow fever. The monkeys were in positive nitrogen balance during the control periods and gained weight. The large increases in nitrogen output, together with a reduced tolerance for the diet during illness, brought about a negative balance during yellow fever accompanied by losses in weight.

The increase in urine nitrogen during the febrile period consisted largely of urea, ammonia, and in some cases, rest nitrogen. The gain in amino-acid, uric acid and creatinine, while large in proportion to the normal excretion, contributed little to the total febrile increment. The rest nitrogen was unusually irregular, sometimes being larger and sometimes smaller during the illness. The urea nitrogen normally formed a rather low percentage of the total nitrogen. In M5 (table 3), it averaged only 49 per cent during the control period when the monkey was being fed 1.2 Gm. of protein per kilogram of body weight daily. While the proportion of urea excreted by the other monkeys studied was higher, it fell short of figures given for human beings on the same relative amounts of protein. Although the ammonia nitrogen in the urine was fairly high, it still did not compensate for the low urea output, and the urea plus ammonia nitrogen was low. For analysis of deaminization and urea formation, of course, urea nitrogen + ammonia nitrogen should be considered as a single component.

The absolute quantities of urea + ammonia in the urine do not usually diminish. In fact, they usually increase. When they do decrease, it is only in the last hours of life when general diminution of nitrogen excretion indicates failure of renal function. Nor does urinary urea + ammonia invariably decrease in proportion to total nitrogen. In the cases presented, the proportion of urea and ammonia fell in monkeys M 1 (table 2), M 2 (table 5) and M 3 (table 1), but rose in M 4 (table 4).

Analysis of the urinary data, therefore, appears to contradict the conclusions drawn from the blood studies. However, the activity of the functions which are under examination, deaminization and urea formation, cannot be estimated from either the rate of accumulation of urea and amino-acids in the blood or from their excretion in the urine, but

only from the rates of production and destruction of these substances in the body. This is measured by the sum of the nitrogen which accumulates in the blood plus that which is excreted in the urine.

Attempts to evaluate the rate of production and destruction of the various nitrogenous constituents have been made and are illustrated in charts 1 and 2. The production (or the resultant of production minus destruction) of any constituent must be represented by the quantity which accumulates in the body plus the amount found in the excreta. It has been assumed for purposes of calculation that at least the most important nitrogenous components, amino-acids and urea, are equally

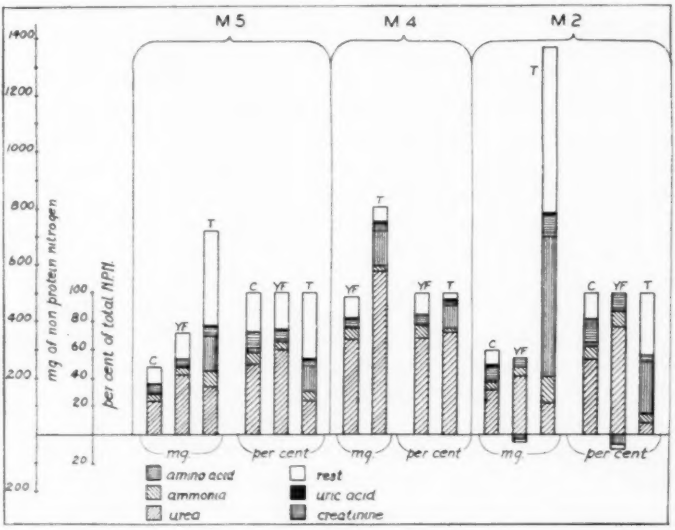

Chart 1.—The actual and relative rates of production of total nonprotein nitrogen and its components during normal control periods, *C*, the course of yellow fever, *YF*, and the premortal stage, *T*, calculated on monkeys M5, M4 and M2 by methods described in the text. Actual rate of production is indicated by the columns enclosed in the brackets marked "mg.," relative rate of production by the columns marked "per cent." In two cases the columns are extended below the base line. In these instances, reductions of the concentrations of the substance in the blood with unchanged or diminished urine excretion indicated actual disappearance, if not destruction, of the particular constituent. This could be expressed graphically only as negative production.

distributed throughout the fluids of the body in the same concentrations in which they appear in the blood and that 70 per cent of the body weight is fluid. The quantity of any nitrogenous component produced in a given interval, therefore, is obtained by adding to the amount found in

the urine a value equal to the change of concentration of the same substance in the blood multiplied by 70 per cent of the body weight. This method of calculation may be illustrated by an example. See Table 7.

The figures used in chart 1 for the terminal period of monkey M 2 were obtained in the following manner. The blood from this monkey on November 24 contained 29 mg. of total nonprotein nitrogen; 13 mg. of urea nitrogen and 8 mg. of amino-acid nitrogen, normal values. It

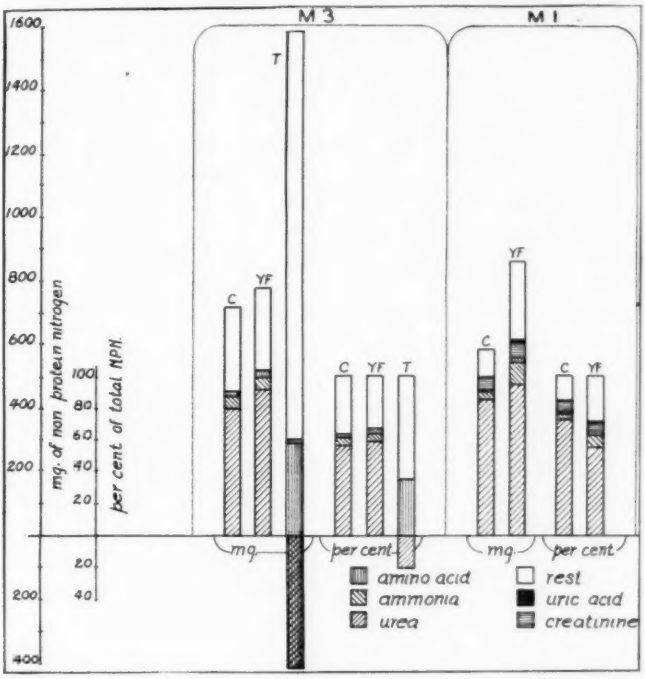

Chart 2.—Same data as shown in chart 1, calculated on monkeys M3 and M1. In the case of M3, the *T* column of actual production has a confusing appearance because the existence of a large negative urea production necessitates extension of the positive amino-acid production below the line to overlap the urea column.

was, therefore, assumed that uric acid and creatinine were also normal, and average normal concentrations for these substances (table 1 of preceding article) were used in the calculations. Blood withdrawn just after death contained significantly larger amounts of all nitrogenous components except urea and uric acid, both of which had fallen slightly. The monkey weighed 3.46 Kg. on November 24, and it is assumed that his weight remained constant until death; 70 per cent of 3.46 Kg. =

2,420 cc. The output of the various nitrogenous constituents is given in table 5. The data from other experiments are given in abstract form in table 8.

Since only insignificant amounts of ammonia nitrogen are found in the blood they have been neglected. The values for total production which are given in column 6 are used in constructing the figures.

TABLE 7.—*Terminal Period in Monkey M2* *

	1 In Blood at Death, Mg. per 100 Cc.	2 In Blood on Nov. 24, Mg. per 100 Cc.	3 Change Between 1-2, Mg. per 100 Cc.	4 Accumulation in Body = 3 × 24.2, Mg.	5 Amount in the Urine in Last 24 Hours,* Mg.	6 Total Production, 4 + 5
Total nonprotein nitrogen..	67	29	38	920	450	1,370
Urea nitrogen.....	10	13	−3	−73	185	112
Ammonia nitrogen.....	..	..	..	....	93	93
Amino-acid nitrogen.....	28	8	20	484	12	496
Uric acid nitrogen.....	0.24	0.29	−0.05	−0.7	9	8
Creatinine nitrogen.....	0.98	0.51	0.47	11.4	59	70
Rest nitrogen.....	28	7	21	508	92	600

* The nature of the results is quantitatively, but not qualitatively, altered if the average excretion for the last two days is used for purposes of calculation.

TABLE 8.—*The Total Production of Nonprotein Nitrogen Constituents During Yellow Fever* *

		Nitrogen Excreted in Urine as				Estimated Accumulation of Nitrogen in Body Fluids as				Total Nitrogen Produced in per Cent of Nonprotein Nitrogen as		
Monkey	Period	Nonprotein Nitrogen, Mg.	Urea + NHs, Mg.	Amino-Acid, Mg.	Rest,† Mg.	Nonprotein Nitrogen, Mg.	Urea, Mg.	Amino-Acid, Mg.	Rest, Mg.	Urea + NHs	Amino-Acid	Rest
M3	Control.....	715	435	13	266	.....	...	...	...	61	2	37
	Yellow fever	774	496	20	258	.....	...	...	...	64	3	33
	Terminal....	25	8	1	16	1,570	−420	700	1,290	−20	44	82
M1‡	Control.....	581	449	7	125	.....	...	...	...	77	1	22
	Yellow fever	860	540	15	304	168§	...	...	...	63	2	35
M5	Control.....	238	139	9	91	.....	...	...	...	58	4	38
	Yellow fever	357	234	7	114	−141§	...	...	...	65	2	33
	Terminal....	237	170	5	62	482	52	121	310	31	17	52
M4	Control.....	457	...	..	...	.....	...	...	...	...	..	..
	Yellow fever	488	375	6	107	.....	...	...	...	77	1	22
	Terminal....	346	278	3	65	458	317	123	18	74	16	10
M2	Control.....	297	180	9	101	.....	...	...	...	62	3	35
	Yellow fever	414	286	6	122	−145	−48	−24	−73	88	−7	18
	Terminal....	450	278	12	160	920	−73	484	508	15	30	49

* For the calculation of the data of M5, average blood nitrogen values for normal period taken from table 1 of the preceding article (footnote 1) were employed.
† Rest nitrogen includes uric acid and creatinine.
‡ Recovered.
§ Not included in total nonprotein nitrogen.

The columns marked *"C"* (control) and *"YF"* (yellow fever) in the figures represent the average daily excretion of nitrogen during the control and yellow fever periods; for the calculation of columns *"T"* (terminal period), urine excretion from the last twenty-four hours only was used.

The assumption that nitrogenous metabolites are equally distributed throughout body fluids is, of course, open to serious question. However, it is the only possible means of approximating the actual production of these metabolites. Madsen,[10] in studies of the distribution of nonprotein, urea and rest nitrogen in the body, found more nonprotein nitrogen in muscle than in blood or other organs, both in health and in disease. Other organs held an intermediate position between blood and muscle. In nitrogen retention, the retained nitrogen was more or less evenly distributed throughout the tissues. Urea, according to Gad-Andresen, appears to diffuse equally throughout the aqueous media of the body.[11] Following hepatectomy, more amino-acid nitrogen has been found in muscle than in blood.[2] It is probable, then, that the increase of amino-acid production is even greater absolutely and in relation to that of urea and total nonprotein nitrogen than the methods of calculation here employed indicate.

From charts 1 and 2 it becomes at once evident that the actual rate of production of urea + ammonia diminished during the terminal period in three of the four fatal cases: monkey M 4 is the only exception. In one instance, monkey M 3, a large quantity of urea appears actually to have disappeared from the body without being excreted in the urine. Amino-acid production (or failure of deaminization) increased in every instance. The proportion of total nitrogen as urea + ammonia in every instance diminished, while that as amino-acid increased greatly.

That these changes are not merely those which accompany febrile illnesses in general can be seen from table 6, which shows the nitrogen metabolism and blood nitrogen of a monkey that died after a fever of unknown etiology. The total excretion of nitrogen in this case is quite as great as that of any of the monkeys with yellow fever. However, throughout the period of observation urea + ammonia makes up about the same proportion of the total urinary nitrogen, and amino-acid excretion remains constant. In the blood, amino-acids and urea remain practically constant during the last twenty-four hours of the disease. The small changes observed correspond in direction and order of magnitude to those which occur immediately after death.[12]

The metabolism studies, therefore, confirm the blood studies. In the terminal stages of yellow fever, and only in the terminal stages, deaminization and urea formation are destroyed or greatly impaired, indicating profound liver damage. To test these functions further, injection of glycin was carried out. The data from these experiments have not been presented, because they showed no departure from the

10. Madsen, S. T.: Acta med. Scandinav. suppl. **7**:318, 1923.

11. Gad-Andresen, K. L.: Biochem. Ztschr. **116**:266, 1921.

12. Wakeman and Morrell (footnote 1, table 4).

normal. The rise of amino-acids in the blood was followed in both instances by an increase of blood urea that was, if anything, greater during yellow fever than in the control periods. It was impossible to calculate the amount of extra nitrogen excreted as urea and ammonia, since there were considerable daily fluctuations in the amounts of urine nitrogen, and because at the times of the second injections (during yellow fever) total nitrogen excretion had begun to fall. However, it was evident that the proportion of the injected amino-acid excreted as such was quite small and approximately the same in both periods. Both monkeys recovered and neither showed any other evidences of loss of deaminizing powers. It is an unfortunate coincidence that neither of them should have developed yellow fever in a malignant form. The experiments afford additional evidence that impairment of deaminization occurs only in the most advanced stages of the disease.

The blood and urine changes occur with great speed as the disease approaches a fatal termination. This is well illustrated by the case of monkey M 3, (table 1), and even better by monkey M 2 (table 3) of the preceding paper.[1] The latter was inoculated on May 26, and on the afternoon of May 28 had a temperature of 104.6 F. At 11:30 a. m. on May 29, when the first blood sample was taken, the monkey was lying down, but he stood up when disturbed. Although the blood sugar at this time was only 34 mg. per hundred cubic centimeters, the blood nitrogen was normal. At 8:30 p. m., the animal was in a state of collapse. It had received no food all day. During this nine hour interval, the blood sugar level had fallen to 18 mg., and the blood nonprotein nitrogen and its fractions had risen to the levels typical of the premortal stage of yellow fever.

Kidney Function.—Complete, or almost complete, anuria is not uncommonly observed during the last day of life, e. g., monkey M 3 (table 1). On the other hand, occasionally, as in the case of monkey M 2 (table 5), there is considerable diuresis. It has already been pointed out [1] that the decreases in the volume of urine may be related to the marked fall of blood pressure which precedes death.

The ability of the kidneys to concentrate nitrogenous metabolites, at least until the premortal period, is demonstrated by the fact that the 2.5 cc. of urine excreted by monkey M 3 (table 1), when the nonprotein nitrogen of the blood was about 66 mg. per hundred cubic centimeters, contained 25 mg. of nitrogen, or 10 Gm. per liter. The lowest concentration occurred in the case of monkey M 5 (table 3), which was 5.4 Gm. of nitrogen per liter. Even this was greater than the average for the control period, 4.9 Gm. The most concentrated urine was that of monkey M 1 (table 2), which averaged 16.5 Gm. of nitrogen per liter during the yellow fever period. Part of the terminal increase of blood nonprotein nitrogen increases are probably to be ascribed to the

extraordinarily high rate of nitrogen destruction that characterizes the disease and taxes the kidneys to the utmost. Undoubtedly, the kidneys are anatomically and functionally damaged; but this injury does not appear to be the chief factor in the production of the most characteristic functional disturbances of the disease. The ability of the kidney itself to produce urine in normal amounts and of normal concentration seems to be retained relatively intact throughout the disease.

SUMMARY

Studies of changes in the nonprotein nitrogenous substances which occur in the blood and urine of *rhesus* monkeys suffering from yellow fever are presented.

The nonprotein nitrogen, amino-acid, urea and rest nitrogen of the blood, increase by considerable amounts during the last hours of life.

Blood creatinine, uric acid and ammonia change little if at all.

Amino-acid increases both in absolute amount and in proportion to the nonprotein nitrogen, while urea decreases in proportion to nonprotein nitrogen. A few cases were found in which there was an absolute decrease in blood urea nitrogen.

These changes were found to be terminal events. No significant alterations occurred during the early stages of the disease or in those monkeys which recovered.

There were no marked alterations in the partition of these substances in the urine.

The changes in the terminal period are shown to be much more pronounced when the accumulation of these substances in the body fluids is considered.

Organic acid and phosphorus excretion usually increased considerably during the disease.

The changes observed were interpreted as resulting from a loss of liver function.

No definite evidence of serious impairment of kidney function was observed, except a terminal anuria probably due to extreme reduction of blood pressure.

THE HAZARD OF INCISION FOR APPARENT QUINSY IN DIPHTHERIA*

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DETROIT

The unhappy result of mistaken diagnosis is usually limited to delay in proper treatment. At times the penalty is greater. During the past few years we have been impressed with the high fatality among patients with diphtheria after surgical incision of the peritonsillar tissues in the belief that the condition was one of peritonsillar abscess.

There are two indications for surgical intervention in diphtheria. Intubation or tracheotomy is necessary when membrane invades the larynx or trachea to such an extent as to interfere with respiration. Extraneous conditions in a patient suffering with diphtheria may require remediation, as acute appendicitis or fracture of the humerus, both of which have been seen in patients with diphtheria.

The disease is primarily a local infection of the mucous membrane of the nose, throat or nasopharynx. At the site of the lesion, a powerful exotoxin is elaborated by the invading infectious agent. This toxin passes to the blood stream by way of the lymphatics draining that region and produces a toxemia of high degree. Toxin carried by the blood may be neutralized in one of two ways. Various tissues of the body may accomplish this end, but only with resulting damage of considerable extent, particularly evidenced by tissues of the nervous and cardiovascular systems. Free toxin before union with tissue may be neutralized by artificially administered antitoxin.

Successful management of faucial diphtheria depends, therefore, on two considerations. Specific antitoxin must be administered early in the course in order that toxin entering the blood stream may be neutralized before union with tissues, thus preventing the degenerative processes which otherwise follow.

The second consideration demands that opportunity for absorption of toxin from the local lesion be limited to a minimum. Trauma evidently favors absorption, because an open lesion offers more favorable conditions than does the relatively intact mucous membrane. Largely because of this consideration, local applications to the throat in diphtheria have fallen into disrepute. The injury to tissue which followed the rather vigorous application of Loeffler's solution and similar prepa-

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* From the Herman Kiefer Hospital, Department of Health.

rations evidently did more harm than good, because of the more ready avenue for absorption of toxin which followed. In present practice, no local applications are used. So thoroughly are physicians impressed with the importance of avoiding local injury that little sympathy exists for the practice, often indulged in, of inducing bleeding of tissues covered by membrane in an attempt to distinguish a diphtheritic from a pyogenic process of the throat. That is no more necessary than feeling the lesions in smallpox. Careful inspection will serve equally well.

If it is important to avoid the minimal injury to tissue resulting from such procedures, it is quite apparent that actual surgical incision of the faucial tissues must constitute a real hazard by favoring absorption of toxin.

Our experience includes no instance in which incision of the peritonsillar space was practiced in a recognized case of faucial diphtheria. Patients in this series were received in the hospital after surgical incision resulting from confusion of the diagnosis of diphtheria with that of quinsy.

PERITONSILLAR ABSCESS SIMULATING DIPHTHERIA

The differentiation of faucial diphtheria and peritonsillar abscess may include errors in both directions; namely, cases of diphtheria may be mistaken for peritonsillar abscess, and instances of abscess may be considered diphtheria. The latter is without great medical importance, as treatment is not altered except in the matter of administering antitoxin, and that does not effect the favorable course of the disease. The only consideration is unnecessary quarantine and isolation.

During 1928 and 1929, 2,977 patients were admitted to Herman Kiefer Hospital with the diagnosis of diphtheria. This diagnosis was confirmed in 78 per cent of the total number. Certain allied conditions account for most of the other cases. Common among these were acute follicular tonsillitis, scarlet fever, Vincent's angina, peritonsillar abscess and diphtheria bacillus carriers; in the latter group, no pathologic factors were evident. The number of patients with quinsy was twenty-nine, a ratio of 1 per cent of all admissions. Total admissions to the service for patients with diphtheria together with final diagnoses are presented in the appended table (table 1).

There is no doubt that a patient with pus formation in tissues centering about the tonsil suffers from a severe type of throat infection. Certain clinical features, however, serve to distinguish this lesion from diphtheria. Patients with quinsy are usually adults, those with diphtheria, children. In contrast to the low temperatures which characterize diphtheria, the temperature in peritonsillar abscess is usually about 102 F. and may be as high as 104 F. In the severe type of faucial diphtheria which might be confused with abscess, it is more likely to be about 100 F.

Patients with peritonsillar abscess complain bitterly of soreness of the throat, while in diphtheria that feature is characteristically in the background. Probably the most important consideration is a unilateral lesion of the throat in the presence of extensive pathologic change. The lesion in diphtheria under comparable conditions would almost surely involve both sides. It is true that peritonsillar abscess may be bilateral, but that is most uncommon; furthermore, we have never observed both spaces affected concurrently. Abscess of one side precedes development on the other. Confusion with diphtheria usually results from the not infrequent presence of a thin, white, often extensive exudate over the

TABLE 1.—*Differential Diagnosis of Diphtheria—2,977 Cases*

Disease	No. of Cases	Total Admissions, per Cent	Disease	No. of Cases	Total Admissions, per Cent
Diphtheria	2,324	78.1	Mercury poisoning	1	0.03
Tonsillitis, acute follicular	300	10.0	Poliomyelitis	1	0.03
Diphtheria bacillus carrier	68	2.3	Contusion of jaw	1	0.03
Scarlet fever	51	1.7	Uremia	1	0.03
Catarrhal laryngitis	42	1.5	Thrush	1	0.03
Vincent's angina	31	1.0	Chronic laryngeal stenosis	1	0.03
Bronchopneumonia	28	0.9	Malnutrition	1	0.03
Peritonsillar abscess	29	1.0	Burns	1	0.03
Nasopharyngitis	13	0.4	Acute lymphatic leukemia	1	0.03
Lobar pneumonia	12	0.4	Chickenpox and whooping cough	1	0.03
Septic sore throat	11	0.4	Pulmonary tuberculosis, chronic	1	0.03
Meningococcus meningitis	6	0.2	Foreign body in esophagus	1	0.03
Measles	6	0.2	Syphilitic ulcer of throat	1	0.03
Diphtheria and measles	4	0.13	Hemorrhagic nephritis	1	0.03
Diphtheria and whooping cough	4	0.13	Tracheobronchial tuberculosis	1	0.03
Rhinitis, acute	3	0.1	Typhoid fever	1	0.03
Chickenpox	3	0.1	Fibroma of nares	1	0.03
Edema of glottis	3	0.1	Diphtheria and German measles	1	0.03
Retropharyngeal abscess	3	0.1	Diphtheria and chickenpox	1	0.03
Whooping cough	3	0.1	Diphtheria, measles and chickenpox	1	0.03
Tuberculous laryngitis	2	0.06	Diphtheria and meningococcus meningitis	1	0.03
Erysipelas	2	0.06			
Mumps	2	0.06			
Stomatitis, aphthous	2	0.06			
Agranulocytic angina	2	0.06			
Traumatic angina	2	0.06			

involved area, which may simulate actual diphtheritic membrane. The accompanying hyperemia is not common in diphtheritic throats. No patient in this series actually suffering from peritonsillar abscess, but reported as having diphtheria, had had the abscess incised previous to admission, because of the well recognized contraindications already emphasized. With two exceptions, all patients of this series whose condition was diagnosed as peritonsillar abscess had four successive negative cultures for Klebs-Loeffler bacilli from material obtained by swabbing the nose and throat. One of these, a girl, aged 11, who gave a history of having had diphtheria, showed a single positive throat culture on her fourth day in the hospital. Cultures taken previously and thereafter were negative. The case was clinically one of abscess. The other patient had a single positive culture from the nose on the first day in the hospital, and thereafter the cultures were bacteriologically negative. The

bacillus proved nonvirulent by virulence tests made on the guinea-pig. These two instances merely serve to emphasize the aphorism applicable to all branches of medicine: in the final analysis, clinical diagnosis must outweigh irrelevant isolated laboratory observations.

Isolation of patients with abscess suggestive of diphtheria is a course to be encouraged and will minimize the unfortunate result that accompanies expectant treatment in diphtheria. By reporting such cases to the hospital for observation and diagnosis, with the full understanding of the patient and his family that such is the status of the case, neither the hospital nor the attending physician incurs criticism, and the patient often benefits. There were no deaths in this group of patients. In many instances, the condition resolved by the use of tightly applied hot packs to the neck. The usual hospital stay was from five to six days. In some instances, it was necessary to incise on account of the localized pus, but this is done to advantage only when the pus has definitely and completely localized.

In the treatment of all abscesses occurring in the course of acute infectious diseases, more satisfactory results are obtained by delaying incision until the pus has definitely localized, with swelling and marked fluctuation by palpation. This would seem to apply to peritonsillar abscess as well as to abscess of the cervical lymph nodes. It certainly is true of retropharyngeal abscess. The rapidity with which the wound subsequently heals more than repays one for the delay in opening the abscess. Complete recovery actually takes place within a shorter period of time. Such delay will, moreover, largely prevent the unfortunate circumstances attendant on making an incision in a diphtheritic throat.

PERITONSILLAR INCISION IN DIPHTHERIA

The mistaken diagnosis of diphtheria in the presence of abscess is without hazard to the patient. That is not true if the condition is diphtheria and is diagnosed quinsy, particularly if surgical measures are instituted. The ordinary simple tonsillar type of diphtheria is not confused with peritonsillar abscess. The possibility of abscess intrudes only when the process extends to involve the nasopharynx, uvula and palate, in which case marked edema and swelling of the adjacent tissues occur. Naturally, incision is most hazardous under these circumstances, because of the extensive diphtheritic process present and the active formation of diphtheria toxin. During 1927, 1928 and 1929, 43 of the 3,260 patients with diphtheria admitted to the hospital had undergone previous surgical incision of the peritonsillar tissues; this is in the proportion of 1:75. Excluding cases of nasal, laryngeal, cutaneous and tonsillar diphtheria in which the possibility of abscess scarcely exists, there was 1 such instance among every 21 cases of faucial diph-

theria in which the differentiation might logically be required. This proportion is unduly great. Surgical procedure in each instance followed an erroneous diagnosis of peritonsillar abscess, later recognized as true diphtheria.

Many of these cases must primarily be of the nasopharyngeal type, in which the membrane is readily discernible only by examination of the posterior surface of the uvula and the back wall of the pharynx. Membrane on the tonsils may be minimal. At any rate, swelling of the faucial tissues is apparently so great that incision of the peritonsillar space is performed in the belief that the condition is one of peritonsillar abscess. There follows a marked production of membrane, which within from twelve to twenty-four hours covers the uvula, soft palate, both tonsils, pharynx and nasopharynx, and even extends over the gingival surfaces. Two cases were seen in which there was membrane even on the buccal mucosa of the cheek. Swelling of the neck is often so extreme as to interfere with respiration. The picture is one of extreme toxemia and constitutes malignant diphtheria, if any case justifies that term. The opening of a raw incised wound at the actual site of active lesions undoubtedly leads to absorption of toxin in a degree not otherwise possible.

Whatever the ultimate outcome after peritonsillar incision, the course is invariably a stormy one. Of those who died, fatal termination usually resulted within the first few days because of the extreme toxemia and associated circulatory failure. A typical example is represented by the following instance.

Case 1.—M. S., a white girl, aged 4½ years, on December 28, developed a sore throat, accompanied by low grade fever and headache. She became worse progressively, the soreness of the throat being more marked on December 31. The left peritonsillar area was lanced on January 1. The patient made no improvement, and on January 2, received 15,000 units of diphtheria antitoxin. Later that day she was admitted to the hospital and received 40,000 additional units. There was, at that time, a purulent nasal discharge, and the glands of the neck were greatly enlarged and moderately tender. There was an extreme bilateral cervical lymphadenitis with periadenitis and edema of the tissues of the neck. The tongue was glazed and dry. A necrotic, grayish red, somewhat hemorrhagic membrane covered the tonsils, uvula, soft palate and posterior pharyngeal wall. The throat was moderately red, with an intense edema of the faucial tissues. A surgical incision extended from the lower part of the soft palate, on the left, down to the tonsil of that side. The borders of the heart were moderately enlarged to the left, and the first heart sound was softened. The fever was 101.8 F. by rectum, the pulse soft and indeterminate. The patient appeared to be extremely toxic; circulatory failure was progressive, and death occurred twenty-four hours after admission

Four patients survived the acute stage of the illness to die late in the disease, about the thirty-fifth day, of multiple postdiphtheritic paralyses and ultimate respiratory failure through dysfunction of the

phrenic nerve. Among the patients who recovered there were also four who had this type of late neurologic condition, but who eventually recovered after a long stay in the hospital, in one instance after ninety-one days.

Case 2.—W. S., a youth, aged 18, on January 14, developed a sore throat accompanied by headache and low grade fever. On January 16, the throat was lanced. No culture was taken for *B. diphtheria*. On January 18, he was given 90,000 units of antitoxin and brought to the hospital. Swelling of the neck was extreme. The membrane in the throat covered practically all visible structures. The temperature was 99.8 F. The patient continued to be extremely ill during the subsequent few days. On January 23, paralysis of the palate developed; by January 26, his condition was extremely poor and his color, ashy gray; a gallop rhythm was heard over the apex of the heart. The systolic blood pressure dropped to 85 of mercury and the diastolic to 55. His condition was precarious for the next week, but thereafter the circulation improved. Unfortunately, successive paralyses of the peripheral nerves involved the palate, the pharynx and the larynx. Eventually,

Table 2.—*Fatality in Diphtheria After Peritonsillar Incision, Compared with the General Fatality from Diphtheria, by Age Groups*

Age, Years	Cases	Deaths	Fatality, per Cent	Fatality in All Types of Diphtheria, per Cent
0 to 4	3	3	100.0	25.0
5 to 9	7	4	57.0	14.2
10 to 14	2	1	50.0	5.3
15 to 19	6	4	67.0	2.0
20 to 29	16	8	50.0	5.1
30 to 39	6	3	50.0	4.0
40 to 49	2	1	50.0	14.2
50 to 59	1	1	100.0	12.5
Total.....	43	25	58.0	12.9

all four extremities were involved, and on February 18, paralysis of the phrenic nerve occurred with consequent respiratory embarrassment. The heart failed under this strain. The patient was irrational on February 23; circulatory failure became more pronounced with a return of gallop rhythm, and marked coldness and numbness of the extremities followed. The patient died on February 24, thirty-seven days after admission to the hospital.

The fatality in these conditions reaches unprecedented levels. Twenty-five of forty-three patients died, giving a fatality of 58 per cent. Among patients with diphtheria of equal severity other than for the factor of incision, whose membrane was just as extensive and who had received antitoxin for the first time at essentially the same stage of the disease, the fatality was 34 per cent. The fatality for diphtheria of all types during the period considered was 12.9 per cent. The hazard of incising the throat in diphtheria would thus appear to be real. It is greater than the actual data indicate, because most of these patients were in advanced age groups, which have a fatality measurably less than that for young children. This is well illustrated by the accompanying table (table 2).

Perhaps the most important deduction from analysis of these forty-three case records of peritonsillar incision in diphtheria is the conclusive demonstration that when antitoxin is given shortly after the incision is made, the fatality is far less than if administration is delayed twenty-four hours or more. Antitoxin was administered to nineteen patients within twenty-four hours of the time of operation, although in some instances in small amounts only. The fatality was 35 per cent, which corresponds almost mathematically with the fatality for patients with diphtheria of approximate severity when incision was not practiced, namely, 34 per cent. The remaining twenty-three patients were given antitoxin at a later period, in all instances twenty-four hours or more after operation, and in one instance three days. The fatality in this group was 77 per cent. These data tend to corroborate our hypothesis that the facility of toxin absorption provided by the open wound accounts for the remarkably high fatality. The incision itself, however, does not markedly affect the ultimate result if the error is immediately compensated by injection of diphtheria antitoxin.

The delayed administration of antitoxin does not of itself account for the greatly increased fatality in these cases. Careful comparison with control cases of equal severity and essentially the same duration in days demonstrates an almost identical fatality in the two groups, provided antitoxin was given within twenty-four hours of the time the surgical incision was made.

So far as can be demonstrated, no increase in fatality through increased opportunity for absorption of toxin follows tracheotomy or the less definite trauma attendant on intubation of the larynx. Why, then, the danger from operative procedure in the peritonsillar region? The answer is that tracheotomy and intubation are performed with the diagnosis of diphtheria definitely made, and administration of antitoxin has preceded or immediately follows the operation. Under similar conditions, we have shown that little added risk accompanies peritonsillar incision. The danger is in delaying the administration of antitoxin. Of minor importance is the fact that lymphatic drainage from the larynx and trachea is less rich than from the pharynx and nasopharynx.

The high fatality in this group occurred despite energetic treatment. Larger amounts of antitoxin than usual were given because of the recognized severity of the illness from which these patients were suffering. Two patients received 90,000 units of antitoxin, five received 80,000, and the usual dose was from 60,000 to 70,000 units. Many patients were given serum intravenously.

The one important recommendation that we would make is that whenever a supposed peritonsillar abscess is incised without delivery of pus antitoxin be administered immediately. If the condition actually

is an abscess, no harm is done; if it proves to be diphtheria, the hazard to life is not markedly increased by incision. Delayed administration of antitoxin beyond twenty-four hours almost invariably results in death.

CONCURRENT DIPHTHERIA AND ABSCESS

Incision of the fauces in diphtheria cannot often be justified by concurrent presence of diphtheria and quinsy in the same patient. It is true that many patients with diphtheria have at the same time double infection of the throat with diphtheria bacilli and some pyogenic invader. Hemolytic streptococci can be isolated from about one fourth of all diphtheritic throats. Many of the severe cases of diphtheria are actually the result of such double infections, and the cervical lymphadenitis, edema and hyperemia of the fauces are due to the action of pyogenic cocci as much as to the diphtheria bacillus itself. Despite the frequency of such secondary infections, only one instance was noted among 3,260 cases of diphtheria in which peritonsillar abscess was associated with acute diphtheria. The abscess subsided without surgical intervention. Abscess complicated convalescence only three times, always after the disappearance of an extensive membrane, once on the seventh day, once on the tenth day and once on the twenty-first day. In no instance was surgical incision necessary.

CONCLUSIONS

Peritonsillar incision in diphtheria causes an extremely high fatality. It happens through confusion in differentiating faucial diphtheria from quinsy. Adults are most commonly concerned, because diphtheria is usually considered in the differentiation of types of sore throat in children, less commonly in angina of adults. High fever is a valuable symptom in distinguishing pyogenic from diphtheritic sore throat. Bilateral swelling of the fauces should suggest diphtheria; unilateral swelling, peritonsillar abscess, although exceptions are noted.

The necessity for incision of peritonsillar abscess is rarely so urgent that in doubtful cases operation may not be delayed until a culture has been taken to determine whether or not the diphtheria bacillus is present.

Whenever a supposed peritonsillar abscess is incised without delivery of pus, we suggest immediate administration of diphtheria antitoxin. If the condition actually is an abscess, no harm is done; if it proves to be diphtheria, the hazard to life is not markedly increased by incision. Delay in the administration of antitoxin beyond twenty-four hours almost invariably results in death.

DEMONSTRATION OF LOCAL IMMUNITY OF THE PERITONEUM BY MEANS OF THE SHWARTZMAN PHENOMENON *

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The conception of local immunity, i.e., the locally increased resistance of a tissue or organ without the participation of the organism as a whole, is not a new one. It has long been recognized that the tissue cell, as the ultimate functional body unit, must be the source from which originate the various protective constituents of normal and immune serums, and that it must be in these cells that the primary changes of the processes known as immunization take place. In support of this view there are both clinical and experimental data; of the former, one notes, for example, the occurrence of crops of furuncles in widely different parts of a patient's skin, one crop healing only to have another crop arise; there the process of limitation and healing of the infected foci is surely not due to any generalized resistance, but rather to local causes. On the experimental side there is, among others, the work of Romer [1] on abrin immunization of the conjunctiva; of Kraus and Volk [2] on the demonstration of the corneal immunity against vaccine virus; of Wassermann and Citron,[3] who demonstrated the lowering of the antibody content of the blood immediately following the removal of the local source of production. Besredka [4] and his followers attempted to explain the phenomena of local immunity on the basis that for each disease there is a certain vulnerable receptive tissue which acts as the normal portal of entry for the virus, and that the protection is conferred on the individual by the local immunization of the specific portal of entry, irrespective of any generalized reaction by other cells in the body. In Europe, especially France, his principles have been applied in the subcutaneous vaccination against anthrax, and also, to a lesser extent, in the prophylactic immunization by mouth against typhoid, paratyphoid, cholera and bacillary dysentery. Gay,[5] in a review of the subject, stated that Besredka's explanations of the

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* From the Laboratories of the Mount Sinai Hospital.

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5. Gay, F. P.: Local or Tissue Immunity, Arch. Path. **1**:590 (April) 1926.

observed facts are not wholly acceptable. He nevertheless affirmed his belief in the existence of a truly localized form of immunity. Steinberg and Goldblatt,[6] and later Herrmann,[7] showed by means of experiments on animals that intraperitoneal injections of streptococcus and colon bacillus vaccine were of definite value in affording protection against fecal soiling of the peritoneum.

In order to demonstrate further the occurrence of local peritoneal immunity, I have employed a phenomenon of local skin reactivity to certain bacterial culture filtrates, first described by Shwartzman[8] in 1928, and confirmed by Hangar[9] and by Ecker and Welch[10] (the latter investigators using the term Shwartzman phenomenon). A rather complete description of this phenomenon is essential in order to understand fully the following experiments. Shwartzman's original work was done with a culture filtrate of *B. typhosus*. The injection of 0.25 cc. of such a toxic substance into the abdominal skin of a normal rabbit will produce no reaction, except perhaps a mild erythema. (It is worthy of note that different areas of skin of the abdomen of the same rabbit may present considerable variations in the intensity of the primary erythema produced by injection into the skin of *B. typhosus* culture filtrate.) If, then, after a period of twenty-four hours one injects as little as 0.01 cc. of the toxic substance per kilogram of body weight intravenously, there is produced within two hours after the injection a blue discoloration at the site of the previous injection into the skin; this discoloration increases rapidly, until at the end of four hours it is extremely pronounced as a dark blue central area with a deep red zone at the periphery; the skin over these hematomas is glossy and smooth.[11] Histologic sections of such a hemorrhagic area show a severe type of hemorrhage and necrosis, with edematous skin, ruptured blood vessels, engorged subcutaneous tissue, extensive leukocytic ingestion and pronounced necrobiosis of these cells both inside and outside of the blood vessels.

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6. Steinberg, B., and Goldblatt, H.: Peritonitis: IV. Production of Active Immunity Against the Fatal Outcome of Experimental Fecal Peritonitis, Arch. Int. Med. **42**:415 (Sept.) 1928.

7. Herrmann, S. F:. Experimental Peritonitis and Peritoneal Immunity, Arch. Surg. **18**:2202 (May) 1929.

8. Shwartzman, G.: Proc. Soc. Exper. Biol. & Med. **25**:560, 1928; J. Exper. Med. **48**:245, 1928; Proc. Soc. Exper. Biol. & Med. **26**:131, 1928; J. Exper. Med. **49**:593, 1929; Proc. Soc. Exper. Biol. & Med. **26**:207, 1928; ibid. **26**:843, 1929; J. Infect. Dis. **45**:283, 1929; J. Exper. Med. **50**:513, 1929; ibid., to be published.

9. Hangar, F. M.: Proc. Soc. Exper. Biol. & Med. **25**:775, 1928.

10. Ecker, E. E., and Welch, H.: J. Exper. Med. **51**:409, 1930.

11. The factors which induced the local skin reactivity are termed "Skin Preparatory Factors" and those involved in production of local hemorrhagic reactions following the intravenous injections, "Reacting Factors."

This reaction may be produced in as high as 85 per cent of rabbits into which injections are made. The intensity and size of the local hemorrhagic reactions are in no way related to the intensity of the erythema produced by the preparatory injection into the skin; animals that show no primary erythema may show a very strong hemorrhagic reaction after intravenous injection, and, conversely, animals that show a marked primary erythema may not react at all to the intravenous dose. That the local trauma produced by the preparatory injection into the skin is not in itself responsible for the localization of the toxic factors introduced intravenously is evidenced when one injects nonspecific irritating substances (turpentine or sterile tryptic digest broth) into the skin in place of *B. typhosus* filtrate. Under such conditions the intravenous injection of *B. typhosus* culture filtrate is not followed by any reactions. Moreover, if instead of injecting the second dose intravenously, one makes another injection into the original area twenty-four hours later, no hemorrhagic necrosis is produced; instead there is only a pronounced reddening of the skin, shown histologically by a marked cellular infiltration without, however, any breaking up of the cells or damage to the blood vessels. Furthermore, if the secondary intravenous injection should be given before fifteen hours or after thirty-two hours, no hemorrhagic reaction results. Thus there is in the phenomenon an entirely new reaction the main features of which are: (*a*) local skin reactivity; (*b*) a definite short incubation period necessary to induce this reactivity (from eighteen to thirty-two hours); (*c*) a short duration of the state of this reactivity; (*d*) the ability to induce local reaction by a single skin injection, and (*e*) the necessity to make the second injection of the toxic substance by the intravenous route. In attempting to modify this last condition, I tried various other ways of introducing the toxic substance into the body.

ATTEMPT TO MODIFY THE SHWARTZMAN PHENOMENON

Methods.—The toxic substances used were prepared as follows: (*a*) Two hundred cubic centimeters of tryptic digest broth were added to 2,000 cc. Erlenmeyer flasks to give a large surface area. The entire growth of one 24 hour old agar slant culture of *B. typhosus* (strain T_L) was suspended in 10 cc. salt solution. Ten cubic centimeters of the suspension was added to each Erlenmeyer flask. The cultures were then filtered through paper and cotton and finally through Berkefeld V candles. The filtrates were tested for sterility and then titrated as to their toxicity by means of the typical Shwartzman reaction. It was found that they would give positive reactions in about 75 per cent of the animals tested if one used as the intravenous dose 2 cc. per kilogram of the body weight of the rabbit. This toxic substance was labeled T_L 527. (*b*) Each of four Kolle flasks was inoculated with the entire growth of one 24 hour old agar slant of *B. typhosus* (strain T_L) which had been suspended in 4 cc. of salt solution. After an incubation period of twenty-four hours, the surfaces of the colonies in each flask were washed with 10 cc. broth and these washings were placed on the sur-

face of Kolle flasks, 3 cc. for each flask. After another incubation period of twenty-four hours the surfaces of the flasks were again washed with 12 cc. of saline each. The cultures were then tested for sterility, filtered through Berkefeld V candles and again tested for sterility. The toxic substance thus obtained was tested by means of the Shwartzman phenomenon and found to give positive reactions in about 80 per cent of the animals, when one used as the intravenous dosage as little as 0.2 cc. per kilogram of body weight of the rabbit. This toxic substance was labeled A_8.

Experimental Work.—Protocol 1: Five-tenths cubic centimeters of filtrate T_L 527 was injected into the upper and lower right and left areas of the skin of the abdomen of eight rabbits. Twenty hours later 12 cc. of the filtrate per kilogram of body weight was fed by mouth to each rabbit. In none of these rabbits was the typical hemorrhagic reaction obtained at the site of the original skin injection. These experiments were repeated, only this time the skin incubation period was shortened to fourteen hours to allow for the slower absorption period from the gastro-intestinal tract. The results, however, were all negative.

Protocol 2: Five-tenths cubic centimeters of filtrate T_L 527 was injected into the upper and lower right and left areas of the skin of the abdomen of eight rabbits. Twenty hours later 8 cc. of the filtrate per kilogram of body weight was injected into the thigh muscles of each of eight rabbits. In none of these rabbits was any hemorrhagic reaction produced. This experiment was also repeated; this time 10 cc. of the filtrate per kilogram of body weight was used, with a skin incubation period of fifteen hours. The same negative results were obtained.

In order to determine whether any substance in the muscle itself was responsible for the prevention of the reaction, extracts of ground muscle were mixed with some of the culture filtrate before the filtrate was injected into the rabbits' abdominal skin; twenty-four hours later the culture filtrate was injected intravenously into these rabbits, and typical hemorrhagic reactions were obtained at the site of the preliminary injection.

Protocol 3: Five-tenths cubic centimeter of culture filtrate T_L 527 was injected into four areas of the abdominal skin of twelve rabbits. Twenty-four hours later each of these rabbits was given 6 cc. of the filtrate (T_L 527) per kilogram of body weight intraperitoneally. Four hours after the intraperitoneal injections had been made, blue discolorations appeared at the site of the previous skin injections in four animals. The discolorations observed rapidly increased until in about six hours the reaction became extremely pronounced, the areas being dark blue in the center with deep red zones at the periphery.

A repetition of the foregoing intraperitoneal experiment, this time using twelve rabbits and allowing only eighteen hours for a skin incubation period, gave positive results (hemorrhagic reactions at the site of the original skin injection) in six rabbits.

Another repetition of the experiment, allowing a skin incubation period of twenty hours and using 3 cc. of the filtrate per kilogram of body weight, gave a positive reaction in one of eight rabbits so treated.

As is seen from the foregoing protocols, the phenomenon of local skin reactivity to *B. typhosus* can be produced by intraperitoneal, as well as by intravenous, injection of the same filtrate.

It was not possible to produce this phenomenon, however, when the second injection was introduced orally or intramuscularly. Just why

these methods failed to produce the reaction is not fully clear, as eventually the toxic substance must be absorbed into the blood stream. As was noted, muscle extract, when mixed with the filtrate and injected into the skin, failed to inhibit the reaction when done by the intravenous method.

THE DEMONSTRATION OF LOCAL TISSUE IMMUNITY

On the basis of the fact noted, namely, that the Shwartzman phenomenon could be elicited by introducing the reacting factors by the intraperitoneal route as well as by the intravenous route, the following experiments were performed.

Method.—One-tenth cubic centimeter of the *B. typhosus* filtrate A_8 was injected into each of four areas of the abdominal skin of thirty rabbits. Twenty hours after the preliminary skin injection each rabbit received 0.5 cc. of this culture filtrate per kilogram of body weight intraperitoneally. (A previous titration had shown that 0.3 cc. of this toxic substance per kilogram was sufficient to produce a positive reaction as evidenced by marked hemorrhagic necrosis at the site of the initial skin inoculation.) It was then attempted to immunize these fourteen positive rabbits against the toxic substance of *B. typhosus*. (For the purpose of immunization the less powerful toxic substance, T_L 527, was used, the minimum titrated intraperitoneal dosage of which was 4 cc. per kilogram of body weight.) These fourteen rabbits were then divided into four groups.

Group 1 (Two Rabbits): These rabbits received no further immunizing dose, but five days after the initial intraperitoneal injection they were again tested in a similar manner (*B. typhosus* culture filtrate A_8, 0.1 cc. into the abdominal skin, followed in twenty hours by 0.5 cc. per kilogram of body weight intraperitoneally) and were found still to react in a markedly positive manner.

Group 2 (Two Rabbits): Three days after the initial intraperitoneal dose, these animals received a second intraperitoneal injection of *B. typhosus* culture filtrate (4 cc. of culture filtrate T_L 527 per kilogram of body weight). After four more days, they were retested in the original manner with the *B. typhosus* culture filtrate A_8 and positive reactions were still obtained.

Group 3 (Eight Rabbits): These animals received an intraperitoneal injection of *B. typhosus* culture filtrate, T_L 527, every third day, the dosage varying from 4 to 6 cc. per kilogram of body weight, in all five injections. Two rabbits died during the course of these inoculations. Three days after the last inoculation the remaining six rabbits were again tested by means of the preliminary skin dose of 0.1 cc. of culture filtrate A_8 followed in twenty hours by an intraperitoneal dose of 0.5 cc. of the same substance per kilogram of body weight. All of these rabbits, which previously had reacted to the intraperitoneal injections in a positive manner, i. e., a hemorrhagic necrosis occurred at the site of the original inoculations, were found to be negative, i. e., they gave no cutaneous reaction. Two days following this, the process was repeated, only this time the second dose of culture filtrate was given intravenously. For the intravenous dose the amount of filtrate A_8 used was 0.2 cc. per kilogram of body weight. Three of the six rabbits showed markedly hemorrhagic necrotic reactions at the site of the cutaneous inoculations; one gave a completely negative response, and the other two showed weakly positive reactions at the site of the preliminary skin injection.

Group 4 (Two Rabbits): These rabbits were given injections in a similar manner to those in group 3, except that they received six intraperitoneal injections of T_L 527. The result of the intravanous retest showed a markedly positive reaction in one, while in the other the abdominal areas remained unchanged.

It is thus seen that of the fourteen rabbits in which a skin reactivity was induced on the introduction of the toxic substance intraperitoneally, the last two groups, which received from five to six intraperitoneal immunizing doses, were rendered negative to a repetition of this reaction. Following this, however, when the toxic substance was introduced into the previously prepared animals intravenously, the typical local hemorrhagic reaction was found to be present in six animals, being markedly positive in four of these, weakly so in two and entirely absent in only two animals.

Demonstration of Local Peritoneal Immunity

Number of Rabbits	Skin Reactions at Previously Prepared Sites, After Intraperitoneal Injection of B. Typhosus Culture Filtrate*	Number of Intraperitoneal Injections of B. Typhosus Culture Filtrate†	Retesting of Rabbits Susceptible to Shwartzman Phenomenon Using B. Typhosus Culture Filtrate, Introduced Intraperitoneally*	Retesting of Rabbits Susceptible to Shwartzman Phenomenon Using B. Typhosus Culture Filtrate, Introduced Intravenously‡
1	++++ (hemorrhagic)	5	0 (negative)	++++
2	++++	5	0	++
3	++++	5	0	++
4	++++	5	0	++++
5	++++	5	0	0
6	++++	5	0	++++
7	++++	6	0	++++
8	++++	6	0	0

* These rabbits received 0.5 cc. of filtrate A_8 per kilogram of body weight.
† These rabbits received from 4 to 6 cc. of filtrate T_L 527 per kilogram of body weight.
‡ These rabbits received 0.2. cc. of filtrate A_8 per kilogram of body weight.

NUMBER OF INJECTIONS NECESSARY TO RENDER PERITONEUM IMMUNE TO B. TYPHOSUS CULTURE FILTRATE

Twelve rabbits, in each of which definite skin reactions had been obtained following the intraperitoneal injection of *B. typhosus* culture filtrate (A_8), were divided into three groups.

Group 1 (four rabbits): Each of these received two intraperitoneal doses of *B. typhosus* culture filtrate at three day intervals (T_L 527, 5 cc. per kilogram of body weight) and were then retested after three days with filtrate A_8, receiving 0.1 cc. of A_8 intracutaneously, followed in twenty hours by 0.5 cc. per kilogram of body weight intraperitoneally. The four rabbits still showed hemorrhagic necrosis at the site of the skin injection, indicating that they had not been rendered immune to the intraperitoneal injections.

Group 2 (four rabbits): The experiments made on group 1 were repeated in all details, save for the fact that three peritoneal immunizing doses were given instead of two. On being retested, only two rabbits gave a positive reaction, while two gave negative reactions.

Group 3 (four rabbits): The experiments made on group 1 were repeated in all details except that four peritoneal immunizing injections were given at three day intervals. On being retested, two rabbits still gave a positive reaction, while two gave entirely negative responses.

It is thus evident that three intraperitoneal injections of *B. typhosus* culture filtrate may be sufficient to render the peritoneum immune to this filtrate. While three and four such injections each rendered 50 per cent of the rabbits immune, previous experiments have shown that five or more injections produced immunity in all of the rabbits so tested.

CONCLUSIONS AND SUMMARY

A modification of the phenomenon of local skin reactivity introduced by Shwartzman is described in this report. This modification consists in the employment of an intraperitoneal instead of an intravenous, injection of toxic substance twenty hours after such a preliminary inoculation into the skin.

By means of this modification, the principle of local peritoneal immunity was demonstrated. Rabbits in which the intraperitoneal injection of *B. typhosus* culture filtrate was able to elicit the Shwartzman phenomenon in previously prepared skin sites were rendered negative to this phenomenon by repeated injections of this culture filtrate intraperitoneally. It was then shown that this skin reaction could still be produced if the reacting factors were introduced intravenously. It must thus be concluded that the immunity produced under these circumstances was of a distinctly local character involving only the peritoneum, and that the entire organism was not yet immune.

It was also determined that between three and four injections of *B. typhosus* culture filtrate were necessary to render the peritoneum immune to this filtrate.

PERNICIOUS ANEMIA

BLOOD REGENERATION DURING EARLY REMISSION *

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The use of liver in pernicious anemia, following the discovery of its therapeutic value by Minot and Murphy,[1] has greatly increased the knowledge on the nature of blood regeneration during remission in this disease. The contributions to this subject of Peabody [2] and of Minot, Murphy, Cohn and their associates [3] have been particularly valuable. According to their observations, the fundamental defect in hematopoiesis during a relapse in pernicious anemia depends on the inability of the primitive red blood cells of bone-marrow to differentiate normally to their adult state. As a result, the hematopoietic tissue of bone-marrow becomes hyperplastic and crowded with megaloblasts. The regenerative activity of bone-marrow being restricted in this fashion, the numbers of red blood cells in the peripheral blood decrease, and many of those present are abnormal. The liver,[4] kidneys [5] and stomach [6] contain a substance which appears to stimulate the maturation of the primitive red blood cells and consequently have a therapeutic action in

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* From the Thomas Henry Simpson Memorial Institute for Medical Research, University of Michigan, Ann Arbor.

1. Minot, G. R., and Murphy, W. P.: Treatment of Pernicious Anemia by a Special Diet, J. A. M. A. **87**:470 (Aug. 14) 1926; A Diet Rich in Liver in the Treatment of Pernicious Anemia, ibid. **89**:759 (Sept. 3) 1927.

2. Peabody, F. W.: The Pathology of the Bone Marrow in Pernicious Anemia, Am. J. Path. **3**:179, 1927.

3. Minot, G. R.; Murphy, W. P., and Stetson, R. P.: Response of the Reticulocytes to Liver Therapy Particularly in Pernicious Anemia, Am. J. M. Sc. **175**:581, 1928. Minot, G. R.; Cohn, E. J.; Murphy, W. P., and Lawson, H. A.: Treatment of Pernicious Anemia with Liver Extract: Effects upon the Production of Immature and Mature Red Blood Cells, Am. J. M. Sc. **175**:599, 1928. Cohn, E. J.; Minot, G. R.; Alles, G. A., and Salter, W. T.: The Nature of the Material in Liver Effective in Pernicious Anemia, J. Biol. Chem. **77**:325, 1928.

4. Isaacs, R.; Sturgis, C. C., and Smith, M.: Treatment of Pernicious Anemia, J. A. M. A. **91**:1687 (Dec. 1) 1928. Minot et al. (footnotes 1 and 3).

5. McCann, W. S. : Effects of Kidney on Blood Regeneration in Pernicious Anemia, Proc. Soc. Exper. Biol. & Med. **25**:255, 1928. Minot and Murphy (footnote 1).

6. Sturgis, C. C., and Isaacs, R.: Desiccated Stomach in the Treatment of Pernicious Anemia, J. A. M. A. **93**:747 (Sept. 7) 1929.

pernicious anemia. When these organs or active preparations of them are fed in sufficient quantity to patients with pernicious anemia, a remission is produced promptly and invariably, and a rapid regeneration of the blood takes place. The regeneration of blood under these circumstances proceeds in such a characteristic fashion that the sequence of events following liver therapy in a particular patient can be predicted with considerable accuracy when the important controlling factors are known.

The purpose in this paper is to discuss certain of these factors which influence the character of blood regeneration in patients with pernicious anemia during liver therapy and to study the manner in which they produce their effect.

THE RETICULOCYTE RESPONSE

In a practical sense, the appearance of increased numbers of reticulocytes in the blood during the first two or three weeks of liver medication, referred to as the reticulocyte response in this paper, is a very important phenomenon. It has some diagnostic significance as, with certain exceptions,[7] a typical reticulocyte response rarely follows liver therapy in diseases other than pernicious anemia. It is of value in prognosis, since a well marked response signifies the onset of a satisfactory remission. Likewise, it may be used to measure the potency of preparations of unknown value and is at present the only reliable standard by which they may be assayed.[8] The extent of the reticulocyte increase also provides a measure of the efficacy of treatment in any given case.

An increase in reticulocytes occurs as a rule only in patients in whom the concentration of erythrocytes in the peripheral blood is less than 3.5 million red blood cells per cubic millimeter of blood before treatment. In patients having more than 3.5 million red blood cells per cubic millimeter of blood, regeneration of blood takes place without an increase in the numbers of reticulocytes in the peripheral circulation.

The important features of the reticulocyte response are its magnitude and the rate at which it takes place. The rate and magnitude of the response are in a sense interdependent, since the numbers of reticulo-

7. Ashford, B. K.: Evaluation of Liver Extract Treatment in Treatment of Anemias of Sprue: Preliminary Note, J. A. M. A. **91**:242 (July 28) 1928. Isaacs, R.; Sturgis, C. C., and Smith, M.: Tapeworm Anemia: Therapeutic Observations, Arch. Int. Med. **42**:313 (Sept.) 1928. Vaughan, J.: Investigation of a Series of Cases of Secondary Anemia Treated with Liver or Liver Extract, Lancet **1**:1063, 1928. Dyke, S. C.: Liver Therapy in Secondary Anemia, ibid. **1**:1192, 1929. Minot et al. (footnote 3, first reference).

8. Minot et al. (footnote 3, second reference).

cytes present at any given time are necessarily smaller, other factors being equal, the more slowly the reticulocytes appear in the blood.

The magnitude of the reticulocyte response is most conveniently expressed either in terms of the concentration of reticulocytes in the blood when their numbers are greatest or as the maximum percentage of erythrocytes containing reticulum observed during the response. The rate of the response is judged from the duration of treatment required to produce the first increase in the reticulocytes in the blood, the maximum number or percentage of reticulocytes observed and the return of the reticulocytes to normal.

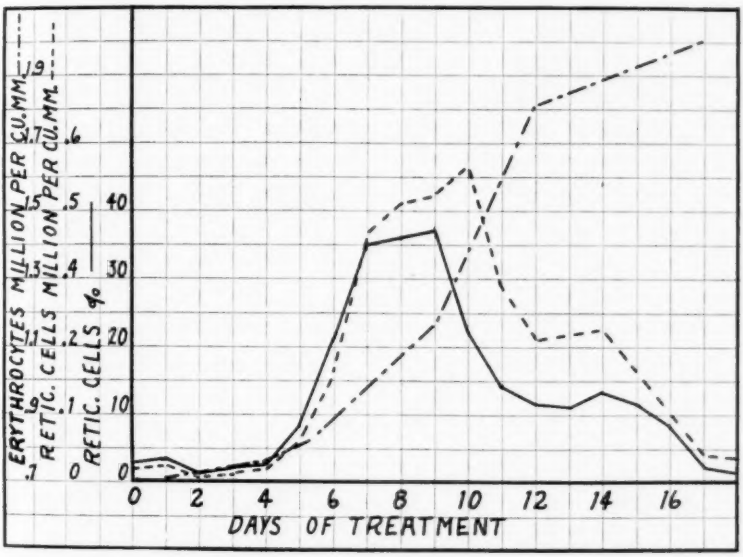

Chart 1.—The changes in the erythrocyte concentration, reticulocyte concentration and reticulocyte percentage in a patient with pernicious anemia during early remission. Treatment consisted of the contents of four vials of liver extract no. 343 (N.N.R.) daily.

When the daily values for the percentage or concentration of reticulocytes during adequate liver therapy are plotted graphically, the resulting curves usually resemble those shown in chart 1. These curves are asymmetrical, the rising slope of the curves representing the period of increasing values being shorter than the downward slope which represents the period of decreasing values, since, as will be shown later, the reticulocytes after being liberated from the bone-marrow probably remain as reticulocytes for a period of two days or more, and consequently tend to accumulate. Ordinarily, the percentage of reticulocytes

first increases above 2 between the second and fifth day of treatment, usually on the third day. A maximum percentage is found ordinarily between the fifth and eighth day. By the tenth to twentieth day of treatment the percentage of reticulocytes has usually fallen to a normal level. As in chart 1, maximum values often are seen on two or more consecutive days, and not infrequently a secondary rise in the percentage of the reticulocytes occurs during the latter part of the response.

The curve representing the actual concentration of reticulocytes is essentially similar to that of their percentage, but, owing to the increasing numbers of erythrocytes during the reticulocyte response, the peak of the curve occurs later than that of the percentage of the reticulocytes.

The form of the curves representing the reticulocyte response is a result of the interaction of the various factors which control the rate and magnitude of the response. To the accustomed eye, considerable information can be gained from an inspection of a characteristic curve as to the dosage of the active liver principle used, the presence of inhibiting factors such as chronic infection and the severity of the anemia before treatment. For example, high pointed curves, short in length, usually occur in patients with low blood counts fed large amounts of liver. Low, irregular, long curves usually indicate insufficient dosage of liver or the presence of inhibiting factors.

MAGNITUDE OF THE RETICULOCYTE RESPONSE

The extensive studies of Minot [3] and his associates and of others [9] have shown that the reticulocyte response in uncomplicated cases of pernicious anemia depends principally on the condition of the hematopoietic tissue in the bone-marrow and the amount of effective liver substance used in treatment. If adequate amounts of liver are used, the magnitude of the response is suggested by them to be directly proportional to the hyperplasia of the bone-marrow and inversely proportional to the concentration of erythrocytes in the blood before treatment. Minot and his associates [10] were able to express mathematically this relationship between the red blood cell count and the magnitude of the response. Their equations representing this relationship are:

$$EpR = \frac{EoR}{1 - R} = 0.73 - 0.2\,Eo$$

Eo represents the red blood cell count before treatment, Ep the red blood cell count at the peak of the reticulocyte response and R the maximum percentage of reticulocytes observed during treatment. The

9. Sturgis, C. C.; Isaacs, R., and Smith, M.: The Treatment of Pernicious Anemia with a Liver Extract, Ann. Int. Med. **1**:983, 1928.

10. Minot et al. (footnote 3, second and third reference).

figures observed in patients under adequate treatment and those calculated by these formulas agree with reasonable accuracy. The equation $EpR = \frac{EoR}{1-R}$ [10] is based on the assumption that EpR = Ep—Eo and that the increase in the value of E between o and p depends solely on the increment of reticulocytes. This idea is open to criticism as there is good evidence that new nonreticulated erythrocytes are produced and that effete erythrocytes are destroyed during this period. Cohn, Minot and their associates recognized this possibility, and in a discussion of this subject [11] stated:

> These conditions can only be expected to obtain provided there has been no significant destruction of red blood cells, nor change in their concentration as a result of redistribution of body fluids. In fact not only must these same conditions obtain, but during the length of time that these three equations yield identical results there can have been no appreciable change of the reticulocytes to adult cells in the blood, nor delivery of adult forms from the bone marrow. In the majority of cases these quantities have not been identical, but their relations to each other are none the less significant.

These two processes must approximately counterbalance one another when the reticulocyte percentage is at its peak, as the equation $EpR = \frac{EoR}{1-R}$ agrees with the observed facts with reasonable accuracy. Evidence presented later in this paper indicates that this is true (chart 5).

Aside from their theoretical interest, these equations are of practical value and can be used as standards for determining the efficacy of treatment and the potency of preparations of unknown value.

Although, as Minot and his associates [8] have suggested, in many respects it is more rational to consider the reticulocyte response in terms of the concentration of reticulocytes rather than their percentage, for the practical use of these formulas it is advantageous to express the relationship between the erythrocyte concentration before treatment and the magnitude of the reticulocyte response in terms of the maximum percentage of reticulocytes to avoid the necessity of making daily estimations of the erythrocyte concentration to obtain the value EpR. This may be accomplished by combining the two equations of Minot and Cohn, thus eliminating the value Ep.

$$EpR = \frac{EoR}{1-R} = 0.73 - 0.2\,Eo$$

$$\frac{EoR}{1-R} = 0.73 - 0.2\,Eo$$

$$R = \frac{0.73 - 0.2\,Eo}{0.73 + 0.8\,Eo}$$

The values of R thus calculated for various values of Eo are shown in table 1, and chart 2. In the chart there is close agreement between the curve representing the calculated values of R and the average

11. Minot et al. (footnote 3, third reference).

observed values of R in selected groups of patients with varying red blood cell counts before treatment, taken from the records of the Simpson Memorial Institute and those of Minot and Cohn and their associates.[8] The figures used in making these average values are selected on the basis that those obtained from patients receiving amounts of liver inadequate to produce an effective response and those from patients in whom some inhibiting factor obviously prevented a typical response were not used in compiling these statistics.

In practice, when adequate treatment is given, the values for the maximum reticulocyte percentage actually observed and those calculated from this equation agree very well. Since the equation represents the average expected value of R, it is not surprising that individual varia-

TABLE 1.—*Expected Average Maximum Reticulocyte Percentage After Adequate Liver Treatment in Pernicious Anemia, with Various Red Blood Cell Counts* *

Red Blood Cell Count in Millions per C.Mm. Before Treatment	Average Maximum Reticulocyte Percentage	Red Blood Cell Count in Millions per C.Mm. Before Treatment	Average Maximum Reticulocyte Percentage	Red Blood Cell Count in Millions per C.Mm. Before Treatment	Average Maximum Reticulocyte Percentage
...	....	1.3	26.5	2.6	7.5
0.1	87.7	1.4	24.3	2.7	6.6
0.2	77.5	1.5	22.3	2.8	5.7
0.3	69.1	1.6	20.4	2.9	4.9
0.4	61.9	1.7	18.7	3.0	4.1
0.5	55.7	1.8	17.1	3.1	3.4
0.6	50.4	1.9	15.6	3.2	2.7
0.7	45.7	2.0	14.1	3.3	2.1
0.8	41.6	2.1	12.9	3.4	1.5
0.9	38.0	2.2	11.6	3.5	0.9
1.0	34.6	2.3	10.5	3.6	0.3
1.1	31.7	2.4	9.4	3.65	0.0
1.2	29.0	2.5	8.4		

* Maximum reticulocyte percentage $= \frac{0.73 - 0.2\ Eo}{0.73 + 0.8\ Eo}$

tions occur slightly above or below the expected values. Such variations may be attributed, in part at least, to the rhythmical daily variations of the reticulocyte percentage,[12] to mechanical difficulties in making extremely accurate estimations of the Eo or R or to individual variations in the condition of the bone-marrow of various patients with similar red blood cell counts.

Values distinctly under those expected from this equation usually indicate inadequate dosage of liver, the use of relatively impotent preparations or the presence of inhibiting factors.

The influence of the dosage of liver on the magnitude of the reticulocyte response has been thoroughly studied by Minot, Cohn and their

12. Porter, W. B., and Irving, H.: Reticulocytosis Produced by Liver Extract: Two, Three and Four Hour Interval Observations, Arch. Int. Med. **44**:502 (Oct.) 1929. Riddle, M. C., and Sturgis, C. C.: The Effect of Massive Doses of Liver Extract on Patients with Pernicious Anemia, unpublished work.

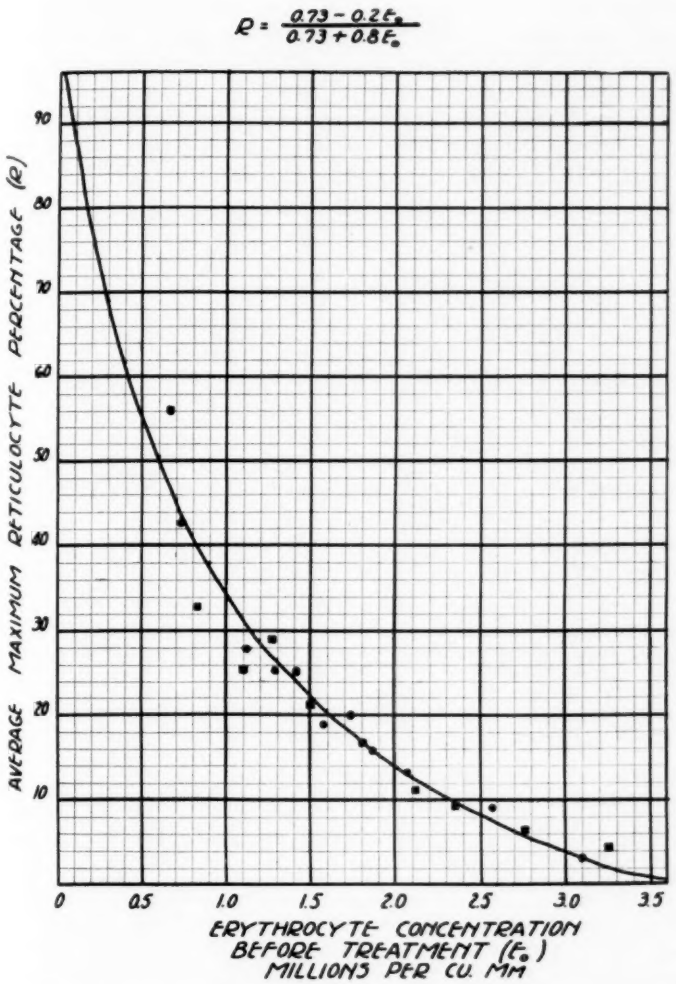

Chart 2.—The average maximum percentage of reticulocytes expected in patients with pernicious anemia after adequate liver extract therapy. The solid squares represent the average values of the maximum reticulocyte percentage observed in sixty-eight patients with pernicious anemia (author's cases) grouped according to the erythrocyte concentration before treatment. The solid circles represent similar values taken for eighty-nine patients observed by Minot and associates (footnote 3, second reference).

associates,[3] who find that the minimum effective daily dose of liver is about 200 Gm. prepared weight. The use of 250 Gm. of liver daily or liver extract no. 343 ("New and Nonofficial Remedies") derived from 300 Gm. of liver usually produces a satisfactory response of the expected magnitude. Larger doses may not increase the magnitude of the response significantly, but when doses smaller than this are used daily, the magnitude of the response is less and is proportional to the dose used.[8]

THE RATE OF THE RETICULOCYTE RESPONSE

It is difficult to express the rate of the reticulocyte response quantitatively. Using the length of treatment necessary to produce a maximum percentage of reticulocytes as an index of the rate of the response, Minot and his associates [8] found that the rate of the response is in a measure proportional to the dosage of liver extract.

TABLE 2.—*Number of Days of Adequate Liver Therapy Necessary to Produce the Beginning and Peak of Reticulocyte Response in Patients with Pernicious Anemia*

First Increase in Reticulocyte Percentage										
Number of days of treatment*	1	2	3	4	5					
Number of patients showing first increase in reticulocyte percentage	4	18	25	14	7					
Maximum Increase in Reticulocyte Percentage										
Number of days of treatment	4	5	6	7	8	9	10	11	12	13
Number of patients showing maximum percentages of reticulocytes	2	11	18	14	14	4	2	1	1	1

* The first day of treatment includes the first twenty-four hours after the initial dose of liver material is given, the second day of treatment the succeeding twenty-four hours, etc.

Table 2 shows the number of days of treatment required to produce the first increase and the maximum increase of the percentage of the reticulocytes in a group of sixty-eight patients with pernicious anemia receiving the contents of 3 vials of liver extract no. 343 (N. N. R.) derived from 300 Gm. of liver or more daily. Between one and five days of treatment was required to produce the first increase in reticulocyte percentage and from four to thirteen days the maximum percentage of reticulocytes in these patients. The first increase in the reticulocyte percentage usually was observed after from two to four days of treatment and the maximum reticulocyte percentage after from five to eight days of treatment.

When these same patients were classified according to the erythrocyte concentration present in the blood before treatment, as in tables 3 and 4, the level of the erythrocyte concentration did not appear to influence the time at which reticulocytes first appeared in increased per-

centage as, on the average, in each group this phenomenon occurred in approximately three days. The maximum reticulocyte percentage, however, appeared slightly earlier on the average the higher the initial erythrocyte concentration before treatment.

TABLE 3.—*Duration of Liver Treatment Required for First Increase of Reticulocyte Percentage* *

Number of Patients	Erythrocyte Concentration Before Treatment, Millions per C.Mm.		Duration of Treatment Before First Increase in Reticulocytes, Days	
	Variation	Average	Variation	Average
11	0.5 - 1.0	0.83	2 - 4	3.4
23	1.0 - 1.5	1.25	1 - 5	2.8
14	1.5 - 2.0	1.80	2 - 5	3.2
15	2.0 - 2.5	2.22	1 - 5	3.1
5	2.5 - 3.5	3.15	2 - 4	3.2

* The dosage of liver medication varied with different patients but each received at least 300 Gm. of liver or an equivalent amount of liver extract daily.

TABLE 4.—*Duration of Liver Treatment Required for Maximum Increase of Reticulocyte Percentage* *

Number of Patients	Erythrocyte Concentration Before Treatment, Millions per C.Mm.		Duration of Treatment Before Maximum of Reticulocytes, Days	
	Variation	Average	Variation	Average
11	0.5 - 1.0	0.83	4 - 10	7.5
23	1.0 - 1.5	1.25	4 - 13	7.2
14	1.5 - 2.0	1.80	5 - 9	7.0
15	2.0 - 2.5	2.22	5 - 11	6.7
5	2.5 - 3.5	3.5	5 - 8	6.6

* The dosage of liver medication varied with different patients but each received at least 300 Gm. of liver or an equivalent amount of liver extract daily.

TABLE 5.—*Relation of Liver Extract Dosage to Rate of Reticulocyte Response*

Number of Patients	First Increase of Reticulocyte Percentage, Days of Treatment		Maximum Increase of Reticulocyte Percentage, Days of Treatment		Liver Extract Dosage, Number of Vials*
	Variation	Average	Variation	Average	
8	5 - 9	6.5	7 - 12	8.9	Weak fractions of unknown potency
8	1 - 5	2.9	5 - 10	8.1	3 vials daily
8	2 - 4	3.0	4 - 8	6.4	4 vials daily
14	2 - 5	3.5	5 - 12	7.3	5 vials daily
21	1 - 5	2.8	5 - 13	7.0	6 vials daily
4	1 - 4	2.7	5 - 7	5.9	9 vials daily
5	2 - 3	2.2	5 - 6	5.2	15–30 vials (single dose)

* One vial of liver extract is derived from 100 Gm. of liver. With few exceptions liver extract no. 343 (N. N. R.) or equally effective preparations were used. In these exceptions, mostly confined to the group receiving 5 vials of liver extract daily, an earlier and less effective preparation of liver extract was used.

In table 5 sixty-eight patients receiving various amounts of liver extract, the dosage for each patient being uniform throughout the period of observation, were classified according to the dosage employed. The rate of the reticulocyte response as judged by the length of treatment necessary to produce the first increase and the maximum increase in the reticulocyte percentage, as a rule seemed to be accelerated by increasing dosages of liver extract. The only obvious exception occurs in the

group receiving liver extract derived from 500 Gm. of liver daily. The liver extract given to this group, in most cases, was not liver extract no. 343 (N. N. R.) but an earlier preparation which was definitely less effective. This may account for the retarded rate in this group. In the patients receiving weak extracts, the rate of the reticulocyte response was obviously delayed. In patients receiving extraordinarily large single doses of liver extract,[13] the rate of the response was extremely rapid, two days being sufficient for the first increase in the reticulocyte response and five days for the maximum percentage values.

These figures indicate that the erythrocyte concentration in the blood of patients with pernicious anemia before treatment has little or no influence on the rate of the reticulocyte response. The rate of the response, however, appears to be accelerated in direct proportion, within certain limits, to the dosage of the effective liver principle employed.

THE RATE OF INCREASE OF THE ERYTHROCYTE CONCENTRATION DURING THE RETICULOCYTE RESPONSE

The foregoing discussion has been concerned with the general character, the magnitude and the rate of the reticulocyte response in large groups of patients. The following discussion has to do with a careful, analytic examination of data gathered from four critically studied patients.

These patients were given a single massive dose of liver extract.[13] Determinations of the reticulocyte percentage were made every four hours, and determinations of the erythrocyte concentration every twelve hours furnished abundant material for mathematical analysis. In these patients an exceedingly rapid and typical reticulocyte response was produced beginning forty-eight hours after the administration of the liver extract, reaching a peak between the fourth and fifth day, and ending between the ninth and tenth day. When plotted graphically, the values of the erythrocyte concentration estimated at frequent intervals during the reticulocyte response usually form a symmetrical, sigmoid curve, as in chart 1, rather than a straight line. The increase in the erythrocyte concentration, at first slow, takes place at an increasingly rapid rate as the peak of the reticulocyte response is approached, then gradually becomes slower toward the end of the response.

The sigmoid character of this curve suggests that the general biologic law of growth and regeneration, represented by the equation $\log \frac{x}{a-x} = ka\,(t-t_1)$, described by Robertson,[14] is applicable to the regeneration of blood during the reticulocyte response in patients with pernicious anemia.

13. Riddle and Sturgis (footnote 12, second reference).

14. Robertson, T. B.: The Chemical Basis of Growth and Senescence, Philadelphia, J. B. Lippincott Company, 1923.

In two of the four patients previously mentioned, the determinations of the erythrocyte concentration were sufficiently accurate for the successful application of this equation; in the remaining two, the data, while more variable than desirable, also seemed to follow this law.

As applied to the increase in the concentration of erythrocytes during the reticulocyte response, the value (x) in this equation represents the increase in the concentration of erythrocytes after any number days of treatment (t). The value (a) is a constant representing the total increase in the erythrocyte concentration during the reticulocyte response, and as blood regeneration takes place during the reticulocyte

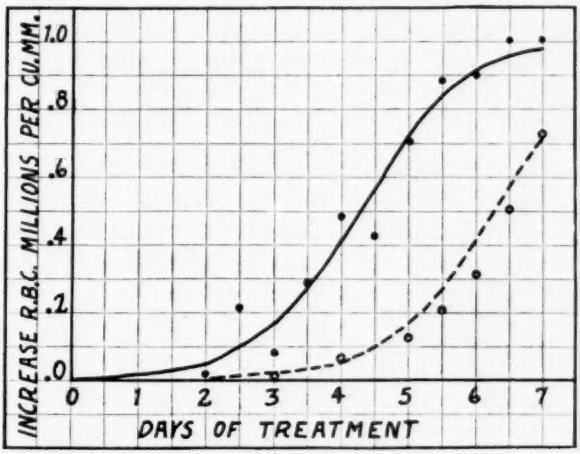

Chart 3.—The increase of the total erythrocyte concentration and of the concentration of nonreticulated erythrocytes in a patient with pernicious anemia after liver treatment. The solid line indicates the increase in the total erythrocyte concentration calculated from the equation $\log \frac{x}{1.02 - x} = 0.54\ t - 4.3)$. The solid circles, the observed values of increase in the total erythrocyte concentration; the broken line, the increase in the concentration of nonreticulated erythrocytes calculated from the equation $\log \frac{x}{1.02 - x} = 0.54\ (t - 6.3)$, and the open circles, the observed values of the concentration of nonreticulated erythocytes obtained by subtracting the concentrations of the reticulocytes from the concentrations of erythrocytes.

response (x) approaches (a) as a limit. The value (t_1) represents the number of days of treatment when $x = \frac{a}{2}$, which usually is near the peak of the reticulocyte response. The value (k) is a constant which is determined for each different application of the formula. This application of Robertson's formula of growth and regeneration to the regen-

eration of blood during the reticulocyte response is illustrated in chart 3. In this patient the formula reads $\log \frac{x}{1.02 - x} = 0.54\ (t—4.3)$. The concentration of erythrocytes in the blood was 0.69 million per cubic millimeter before treatment. At the end of the reticulocyte response, the erythrocyte concentration was 1.71 million per cubic millimeter. The difference between these two values or 1.02 is the value (a) in the equation. The value of (k) which best agreed with the data in this case was 0.53 so (ka) equals 0.54 in the equation. The peak of the reticulocyte response was observed to occur 104 hours or 4.3 days after the administration of liver giving the value for (t_1) as 4.3. In chart 3, the increase in erythrocyte concentration observed at twelve hour intervals and those calculated according to the formula appear to agree very satisfactorily considering the degree of accuracy with which red blood cells may be counted. In the same chart the increase in the numbers of nonreticulated erythrocytes, obtained by subtracting the observed concentration of reticulocytes from the observed concentration of erythrocytes, is compared with the values obtained from the equation $\log \frac{x}{1.02 - x} = 0.54\ (t—6.3)$, which differs from the preceding equation only in the value for (t_1) which is in this case increased by two days to 6.3. The concentration of nonreticulated erythrocytes thus appears to increase in a manner similar to the total concentration of erythrocytes but later by approximately two days in this patient.

RATE OF LIBERATION OF THE RETICULOCYTES INTO THE BLOOD DURING THE RETICULOCYTE RESPONSE

Since reticulocytes are continually being liberated from the bone-marrow into the blood and while in the blood apparently are being transformed constantly into mature erythrocytes, no accurate estimate of the number released from the bone-marrow in a given time can be made unless the time required for the reticulocyte to lose its reticular material and become a mature erythrocyte is known.

A general idea of the rate of the liberation of reticulocytes into the blood can be obtained from the changes in their concentration in the blood from time to time. If the concentration is increasing it may be assumed that the rate of their release into the blood is greater than the rate of their transformation into mature erythrocytes and that they are accumulating in the blood. The reverse is true as their concentration decreases in the blood.

The data shown in chart 3 is evidence that in this patient the reticulocytes became nonreticulated after being approximately two days in the blood. With this knowledge and with the data on the reticulocyte concentration at frequent intervals, it was possible to apply Robertson's formula again. This time the value (x) represents the total number

of red blood cells liberated into the blood as reticulocytes, within a certain number of days of treatment (t) including both those still retaining reticulum and those transformed into mature erythrocytes. If the reticulocytes in this patient became mature in two days the concentration of reticulocytes at a given time (t), should equal the increase in the value of (x) between (t—2) and (t); in other words, the number of reticulocytes released during the preceding two days. When the various values of (x) for various values of (t) were obtained in this way from the observed values for the reticulocyte concentration, it was possible after a number of trials to find that the values for the constants (a)

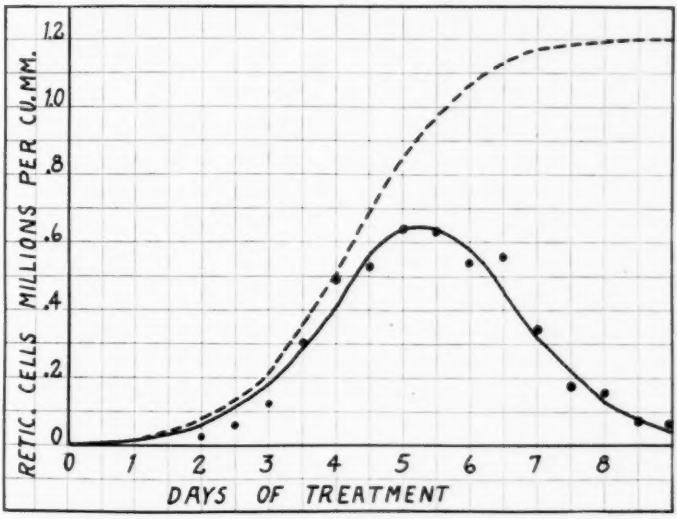

Chart 4.—The concentration of reticulocytes in a patient with pernicious anemia. The broken line indicates the values of the numbers of reticulocytes per cubic millimeter of blood liberated from the bone-marrow calculated from the equation $\log \frac{x}{1.2-x} = 0.54\ (t-4.3)$; the solid line, the values of the concentration of reticulocytes after any number of days of treatment (t) obtained by subtracting values of x at t—2 from values of x at t; the solid circles, the observed values of the concentration of reticulocytes.

and (k) which best satisfied the data at hand were 1.2 and 0.45, respectively. The value of t_1) from the previous use of the equation was known to be 4.3. Chart 4 presents the results obtained in this manner. The formula used is $\log \frac{x}{1.2-x} = 0.54\ (t-4.3)$. The results calculated from this equation are plotted as a broken line. The curve drawn as a solid line represents the values of (x) at (t) minus the values of (x)

at (t—2) and according to the reasoning employed should correspond to the curve of the reticulocyte concentration. The close agreement between the latter curve and the observed values for the concentration of the reticulocytes is obvious. This fact is further evidence that in this patient the reticulocytes lost their reticulum and became mature erythrocytes after being in the blood for two days. The value of (a) was 1.2 in this formula. In the formula for the increase in the erythrocyte concentration, the value of (a) was 1.02. This difference may be attributed to the destruction of effete red blood cells during the period involved. When the values of (x) representing the increase in the erythrocyte concentration obtained from the first equation $\log \frac{x}{1.02 - x}$

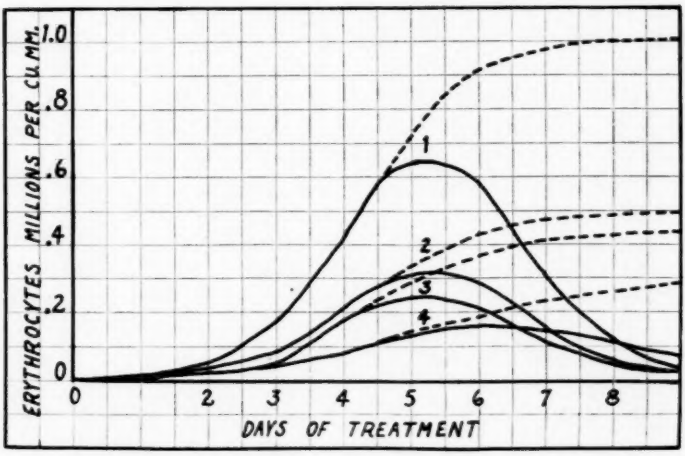

Chart 5.—The concentration of reticulocytes and the increase in concentration of erythrocytes during the reticulocyte response in four patients with pernicious anemia given a single massive dose of liver extract calculated from Robertson's equation for growth and regeneration. The solid lines indicate concentrations of reticulated erythrocytes which equal $(x_t - x_{t-2})$ in the following equations: patient 1, $\log \frac{x}{1.2 - x} = 0.54$ (t—4.3); patient 2, $\log \frac{x}{0.6 - x} = 0.50$ (t—4.3); patient 3, $\log \frac{x}{0.5 - x} = 0.48$ (t—4.3); patient 4, $\log \frac{x}{0.45 - x} = 0.3$ (t—5.3). The broken lines show the increase in total erythrocyte concentrations derived from the following equations; patient 1, $\log \frac{x}{1.02 - x} = 0.54$ (t—4.3); patient 2, $\log \frac{x}{0.5 - x} = 0.50$ (t—4.3); patient 3, $\log \frac{x}{0.43 - x} = 0.48$ (t—4.3); patient 4, $\log \frac{x}{0.3 - x} = 0.3$ (t—5.2).

The erythrocyte concentration in millions per cubic millimeter before treatment was as follows: patient 1, 0.69; patient 2, 1.43; patient 3, 1.87; patient 4, 2.82. The dosage of liver extract used in each case was as follows: patients 1, 2, 3, a single dose of the contents of thirty vials of liver extract; patient 4, a single dose of the contents of fifteen vials of liver extract.

$= 0.54$ (t—4.3) and the value of $x_c - x$ (t—2) from the equation $\log \frac{x}{1.2 - x} = 0.54$ (t—4.3) representing the concentration of reticulocytes at t, between 0 and 4.3 days, are compared, they appear to be identical up to the peak of the reticulocyte response. This seems to indicate, as has been suggested before, that up to the peak of the response, the numbers of reticulocytes transformed into mature erythrocytes equals the numbers of effete erythrocytes destroyed and explains why the equation $EpR = \frac{EoR}{1-R}$ of Minot and his associates [10] can be applied so satisfactorily.

Similar applications of Robertson's equation were made in the other three patients with similar results. In all of them the data indicated that the reticulocytes once liberated into the blood were transformed into mature erythrocytes in a two day period. Thus the rate of the reticulocyte response was the same, although the erythrocyte concentration before treatment varied in these four patients between 0.69 and 2.82. Chart 5 shows the curves representing the reticulocyte concentration and increase in the concentration of the erythrocytes calculated for these four patients in the manner already described and illustrates the point made previously that at the peak of the response the increase in the reticulocyte concentration equals the increase in the erythrocyte concentration.

Without further confirmation, this method of analysis cannot be said to have wide application. However, it offers a new method for approaching the solution of problems concerned with blood regeneration otherwise difficult of solution. Furthermore, it suggests that, under the circumstances, blood regeneration in these patients can be described by the formula of growth and regeneration used by Robertson.[14]

DIFFERENTIATION OF RETICULOCYTES IN THE BLOOD

In the patients previously discussed, under the intense stimulation of a very large dose of liver extract blood regeneration was unusually rapid, the entire reticulocyte response requiring less than ten days for its completion, a shorter period than ordinarily is required when smaller daily doses of the liver principle are used. It is probable that the transformation of the reticulocytes to mature erythrocytes, which in this case required only two days, requires longer in patients in whom the stimulus to blood regeneration is less intense. On the other hand, it also is likely that in normal persons and in persons less anemic than these patients the reticulocytes are retained in the bone-marrow for a longer time and are developed to a stage approaching nearer to maturity before their liberation and may consequently require less time in the blood to lose their reticulum. This matter appears to require special consideration in each individual case, as so many variable factors are involved.

In this discussion it has been assumed, and the evidence strongly favors the assumption, that once released into the blood reticulocytes develop into mature erythrocytes. As an alternative, it might be suggested that this does not necessarily occur, the reticulocytes being destroyed as such in the blood. The correlation between the numbers of reticulocytes appearing in the blood and the increase in the numbers of red blood cells during the reticulocyte response is so close that the destruction of significant numbers of reticulocytes seems improbable.

Furthermore, the changes in the microscopic appearance of the reticulocytes from day to day during the reticulocyte response indicates that while in the blood the reticulocyte gradually loses the substance which stains as reticular material. The reticulocyte population in any blood is not homogeneous. During periods of active blood regeneration, all gradations between normoblasts with heavy reticulation and mature erythrocytes may be found in a single drop of blood. The heaviest reticular material usually is seen in cells in which fragments of the normoblast nucleus are present. When only small strands of reticular material are present, the cell is indistinguishable from normal erythrocytes, except for the presence of the reticular material. From this evidence it appears that the reticular substance appears in large quantities as the normoblast loses its nucleus and that as the reticulocyte becomes increasingly mature the reticular substance is gradually lost, a mature erythrocyte being formed. This relationship between the degree of maturity of the red blood cell and its content of reticular material has been noted by various investigators.[15]

Characteristically, during the first few days of the reticulocyte response as these cells are rapidly increasing, the majority of the reticulocytes are heavily laden with reticular material. As the percentage of reticulocytes declines toward the end of the response, reticulocytes containing but little reticular material predominate. In other words, heavy reticulation is more frequent when the number of reticulocytes being released into the blood each day exceeds the number which have been present in the blood for a longer time. When the reverse is true, faintly reticulated cells are more frequent. This fact is shown in table 6 in which the reticulocytes are classified according to the amount of reticular material they contain. From the mathematical data presented, it was possible to compare the percentages of reticulocytes in this patient present each day which had been released into the blood between

15. Seyfarth, O., and Jurgens, R.: Untersuchungen über das Verhalten der vitalgranulierten roten Blutzellen (Retikulocyten) bei Embryonen und Neugebornen, Virchows Arch. f. path. Anat. **266**:676, 1927. Davidson, S.: McCrie, J. G., and Gulland, G. L.: The Treatment of Pernicious Anemia with Liver and Liver Extracts, Lancet **1**:847, 1928. Schilling, V.: Wirkungen und Ausblicke der Lebertherapie bei Anaemia Perniciosa, Klin. Wchnschr. **1**:882, 1928.

twenty-four and forty-eight hours before with the degree of reticulation. From this comparison, it was deduced that reticulocytes with moderate to heavy reticulation had been liberated into the blood during the preceding twenty-four hours, those with faint reticulation having been present in the blood between twenty-four and forty-eight hours (table 6).

A mathematical analysis also was performed in the manner previously outlined in two patients who received daily the contents of three and four vials of liver extract no. 343 (N.N.R.), respectively. In the first patient the duration of the reticulocyte response, from the start of treatment until the end of the response, was twenty-four days. In this patient, during the first part of the reticulocyte response, according to the data, four days was required for the transformation of the

TABLE 6.—*Density of Reticular Substance in Reticulocytes in Relation to Age of Reticulocyte*

Days of Treatment	Percentage of Erythrocytes Containing Reticular Substance	Percentage of Reticulocytes in the Blood with Various Densities of Reticular Substance (Observed) *				Percentage of Reticulocytes Released into the Blood Between 24 and 48 Hours Before (Estimated)†
		++++	+++	++	+	
2	2.5	20	34	26	20	22
3	16.1	22	22	28	28	25
4	42.2	12	21	35	32	31
5	45.4	4	17	36	43	44
6	27.6	4	11	24	61	61
7	20.0	1	4	21	74	72
8	11.7	0	5	19	76	75
9	4.7	4	5	12	79	76

* ++++ = very dense reticular substance practically filling the reticulocyte; +++ = dense reticular substance in form of band, wreath or plaque; ++ = scattered clumps of reticular substance present; + = scattered strands of reticular substance.

† Estimated from the equation $\log \frac{x}{1.2-x} = .54\ (t-4.3)$ (see chart 4).

reticulocytes in the blood into mature erythrocytes and during the latter part of the response three days was required. In the second patient the time required to complete the reticulocyte response was eighteen days, and for the disappearance of reticular material from the reticulocytes in the blood, three days. In these two patients in whom the reticulocyte response covered a longer period of time than in the patients previously discussed, the time required for the maturation of the reticulocytes in the blood into mature erythrocytes was proportionately longer. The rate of the maturation of the reticulocytes in the blood seemed to be related to the dosage of liver extract used.

Pepper,[16] studying blood in vitro at body temperature, observed that the reticulation of reticulocytes began to decrease perceptibly after

16. Pepper, O. H. P.: Vitally Stainable Reticulation and Chromatic Granules in Erythrocytes in Vitro, Arch. Int. Med. **30**:801 (Dec.) 1922.

eighteen hours and disappeared completely after seventy-two hours, a fact of interest in the present discussion.

In the blood of normal persons the reticulocytes contain only small amounts of reticular substance as a rule. If the observation that a lessened amount of reticular material is a sign of approaching maturity is sound, this would suggest that during the normal regeneration of blood the reticulocytes lose most of their reticular material before they are released from the bone-marrow, and that once present in the blood stream they lose the small amount of reticular material remaining within a relatively short time.

PHYSIOLOGIC CHANGES ACCOMPANYING BLOOD REGENERATION DURING REMISSION

The rapid regeneration of blood during the early part of remissions induced by the active principle contained in liver, recent investigations have shown, is accompanied by certain important physiologic changes in the blood and excreta. The amount of bilirubin in the blood, characteristically increased during relapse in pernicious anemia, rapidly decreases to a normal or subnormal level with the onset of remission.[17] Iron, although used in large quantities for the manufacture of hemoglobin, is excreted in amounts greatly in excess of the iron intake during early remission.[18] It is well known that an iron deficiency is not present during relapse, that iron is stored in considerable quantities in the body despite the anemia, and that iron therapy is illogical in pernicious anemia. During the early part of remission, the blood sugar during fasting becomes lowered,[19] the restoration of appetite possibly being related to the relative hypoglycemia thus produced. Endogenous uric acid is manufactured and excreted in large quantities during the period of the reticulocyte response.[20] The amount of endogenous uric acid excreted in excess of the normal excretion is proportional to the increase in the numbers of red blood cells in the blood. From the figures given in table 7, it appears that on the average with the increase of a million red blood cells per cubic millimeter of blood approximately 10 Gm. of endogenous uric acid is excreted in the urine. The destruction of normoblast nuclei appears to be the source of this endogenous uric acid, and

17. Murphy, W. P.; Monroe, R. T., and Fitz, R.: Changes in the Composition of Blood in Pernicious Anemia, J. A. M. A. **88**:1211 (April 16) 1927.

18. Riecker, H. H., and Winters, M. E.: Serum Iron Studies, Proc. Am. Soc. Clin. Investigation, J. Clin. Investigation **7**:497, 1929.

19. Riddle, M. C.: The Blood Sugar During Remission in Pernicious Anemia, unpublished work. Blotner, H., and Murphy, W. P.: The Effect of Liver on the Blood Sugar Level, J. A. M. A. **92**:1332 (April 20) 1929.

20. Riddle, M. C., and Sturgis, C. C.: The Endogenous Uric Acid Metabolism in Pernicious Anemia, Proc. Am. Soc. Clin. Investigation, J. Clin. Investigation **7**:498, 1929. Riddle, M. C.: The Endogenous Uric Acid Metabolism in Pernicious Anemia, J. Clin. Investigation **8**:69, 1929.

it seems likely that the uric acid of endogenous origin normally excreted in the urine is derived principally from this source. This idea has confirmation in the researches of Krafka [21] on the excretion of endogenous uric acid in the Dalmation coach-hound during blood regeneration after hemorrhage and the observations of Isaacs [22] on the uric acid metabolism in polycythemia.

The endogenous uric acid metabolism, being so intimately related to blood regeneration, is a reasonably reliable biochemical index of blood production during early remission. The rise in the concentration of uric acid of endogenous origin in the blood and its excretion in increased amounts in the urine apparently have the same significance as the increase in the numbers of reticulocytes in the blood, and can be detected earlier, as changes in the endogenous uric acid metabolism usually

TABLE 7.—*Relation of Endogenous Uric Acid Excretion to the Increase in the Erythrocyte Concentration During Remission in Pernicious Anemia*

Patient	Days of Treatment	Endogenous Uric Acid Excretion, Gm.	Increase in Erythrocyte Concentration Million per C. Mm.	Uric Acid Excretion per Million Increase in Erythrocyte Concentration, Gm.
1	8	5.60	0.74	7.6
2	21	20.15	2.39	8.5
3	42	33.59	3.66	9.2
4	14	10.09	1.09	9.3
5	14	10.15	1.04	9.8
6	19	19.73	1.95	10.1
7	12	7.44	0.71	10.5
8	9	8.80	0.79	11.2
9	14	10.71	0.95	11.3
10	8	4.90	0.43	11.4

precede similar changes in the reticulocyte concentration in the blood by one or two days. According to Muller and his associates,[23] there is also an increase in the cholesterol and lecithin content of the blood plasma which accompanies the reticulocyte response during early remission.

COMMENT

According to the evidence presented in this paper, the conclusion may be drawn, in agreement with the observations of Minot and his associates,[24] that aside from the presence of inhibiting complications in patients with pernicious anemia, the two principal factors that regulate the reticulocyte response to liver therapy are the condition of the hema-

21. Krafka, J.: Endogenous Uric Acid and Hematopoiesis, J. Biol. Chem. **83**:409, 1929.

22. Isaacs, R.: Pathologic Physiology of Polycythemia Vera, Arch. Int. Med. **31**:289 (Feb.) 1923.

23. Muller, G. L.; Castle, W. B.; Goode, E., and Rose, M.: Relation of Cholesterol and Lecithin to Remission in Pernicious Anemia, Proc. Soc. Exper. Biol. & Med. **25**:567, 1927-1928.

24. Minot et al. (footnote 3, first and second references).

topoietic tissue of the bone-marrow before treatment and the amount of the active principle contained in liver used in treatment.

The concentration of erythrocytes in a patient's blood before treatment, being inversely proportional to the degree of bone-marrow hyperplasia, is a satisfactory index of the extent of the hyperplasia and consequently can be used in mathematical formulas by which the average magnitude of the reticulocyte response to adequate liver medication in patients can be predicted with reasonable accuracy. The condition of the bone-marrow before treatment apparently does not influence directly the rate of differentiation of the primitive red blood-forming cells of bone-marrow to their more mature erythrocyte form after liver is administered. The reticulocyte response, figures show, occurs approximately at the same rate in patients with varying degrees of anemia when similar doses of liver or liver extract are used. Indirectly, the condition of the bone-marrow before treatment may modify the velocity of the reticulocyte response, as the data at hand suggest that the rate of the release of blood cells from the hematopoietic tissue of bone-marrow into the blood is proportioned both to the rate at which they are differentiating and to the numbers released into the blood in unit periods of time. The dosage of liver or liver extract used appears to influence directly the rate of cellular differentiation in the hematopoietic tissue of bone-marrow, and consequently influences in a direct manner the rate at which reticulocytes are liberated into the blood and the rate of the reticulocyte response. The magnitude of the reticulocyte response, being principally determined by the numbers of primitive red blood-forming cells in the bone-marrow available for transformation into reticulocytes and for their liberation from the bone-marrow into the blood, is influenced only in an indirect manner by the dosage of liver principle employed. The numbers of reticulocytes that accumulate in the blood at the peak of the response depend on the numbers formed in bone-marrow and released into the blood and the length of time reticulocytes remain as such in the blood. The magnitude of the response thus depends indirectly on the rate at which the reticulocytes are formed from the more immature cells of the bone-marrow. This effect is modified to a certain extent by the probability that if the rate of differentiation of the primitive red blood cells of bone-marrow into reticulocytes is slow, the differentiation of reticulocytes into erythrocytes in the blood also will be slow, and they will therefore tend to accumulate in greater numbers in the blood.

According to these theoretical considerations of the influence of the condition of the bone-marrow and the dosage of the liver principle on the rate, magnitude and character of the reticulocyte response, the rate and magnitude of the response are to a certain extent interdependent

and modify one another in the complex regenerative processes associated with blood formation during early remission after liver therapy in pernicious anemia.

The general law of biologic growth and regeneration widely applied by Robertson [14] to various problems of growth and regeneration in plants and animals appears to be applicable to the phenomenon of the reticulocyte response. It should be emphasized that the application of this formula can be attempted only when the data are accurate and abundant and the reticulocyte response is typical and uniform. The use of this formula when it can be applied offers a new method for the solution of problems concerned with blood regeneration otherwise difficult of solution.

With the aid of this formula, an estimate was made of the length of time required for the reticulocytes in the blood of four patients given a massive dose of liver extract to become differentiated to their mature erythrocyte form. In these patients, blood regeneration took place at an unusually rapid rate as a result of the use of an extremely large amount of liver extract. The reticulocytes seemed to reach maturity and become indistinguishable from other erythrocytes two days after their liberation from the bone-marrow. In two other patients who received daily the contents of three and four vials of liver extract, respectively, the reticulocyte response developed at a slower rate, the differentiation of erythrocytes from reticulocytes in the blood requiring from three to four days.

The relations noted between the amount and density of reticular substance and the degree of maturity of the reticulocytes supports the evidence, derived from Robertson's formula, that the reticulocytes in the blood of the four patients given a massive dose of liver extract required two days in the blood stream for differentiation into mature cells. This evidence corroborates what has been noted by others,[15] namely, that a large amount of reticular substance in a reticulocyte is a sign of relative immaturity of the red blood cells and that a scanty amount of this material is a sign of approaching maturity. Early in the response to liver therapy, as the reticulocytes increase, a greater proportion of them contain more reticular material than after the peak of the response has been reached, which indicates that early in the response the reticulocytes are liberated from the bone-marrow in a more immature form than later, that is, near the end of the response. Also, the proportion of reticulocytes with a small amount of reticular substance becomes greater as the reticulocyte response proceeds. A comparison of the degree of reticulation with the daily changes in the concentrations of reticulocytes and mature erythrocytes indicated that the reticulocytes containing heavy reticular material present at any given time had appeared in the blood

during the previous twenty-four hours. The faintly reticulated reticulocytes seemed to disappear within the succeeding twenty-four hours as they became mature in these four patients in whom the reticulocyte response was unusually rapid. When the length of time that the reticulocytes persisted as such in the blood was known, it was also possible with Robertson's equation to reproduce accurately in mathematical terms the curves representing the concentration of reticulocytes.

SUMMARY

1. From evidence collected from a group of sixty-eight patients with pernicious anemia undergoing remission as a result of liver treatment and from a consideration of the theoretical factors that influence blood regeneration under these circumstances, it appears that the magnitude of the reticulocyte response is governed directly by the extent of the bone-marrow hyperplasia during relapse, and that the rate of the reticulocyte response is governed directly by the dosage of the active principle contained in liver.

2. As the magnitude and the rate of the reticulocyte response are to a certain extent interdependent, the condition of the bone-marrow apparently influences indirectly the rate of the response, and the dosage of liver used influences indirectly the magnitude of the response.

3. The percentage of reticulocytes expected, on the average, at the peak of the reticulocyte response can be formulated in terms of the concentration of erythrocytes before treatment. The equation $R = \frac{0.73 - 0.2\ Eo}{0.73 + 0.8\ Eo}$ which represents this relationship can be used in determining the efficiency of treatment and in testing the potency of extracts of unknown value.

4. For four patients whose collected data were satisfactory for mathematical analysis, the blood regeneration was formulated according to an equation used by Robertson to express a general law of biologic growth and regeneration.

5. When the rate at which the reticulocytes in the blood of these patients were transformed into mature erythrocytes was estimated by this equation, the curve representing the concentration of reticulocytes during the reticulocyte response could be duplicated mathematically.

6. It was estimated that in these patients two days were required for the differentiation of reticulocytes in the blood into mature erythrocytes. In two other patients in whom the stimulus to regeneration of blood was less intense, it was estimated that between three and four days were required for the maturation of reticulocytes in the blood to their mature form.

7. A correlation of the relative maturity of the reticulocyte with the amount of reticular material it contained showed that, under the circumstances present, reticulocytes containing small amounts of reticular material disappeared during the following day.

8. When the amount of reticular substance in a recticulocyte was used as a standard for estimating its degree of maturity, it was found that during the early part of the reticulocyte response reticulocytes were liberated in an earlier stage of development than toward the end of the response.

9. The rate at which reticulocytes are liberated from the bone-marrow into the blood appears to be a factor of importance which influences the rate and magnitude and velocity of the reticulocyte response. The rate of the liberation of the reticulocytes into the blood seems to be proportional to the rate at which they are formed from the more primitive red blood cells within the bone-marrow and to the numbers of reticulocytes being formed there.

10. An increase of a million red blood cells per cubic millimeter of blood is accompanied by an excretion of approximately 10 Gm. of endogenous uric acid in patients with pernicious anemia during early remission.

RED BLOOD CELL SIZE IN ANEMIA

ITS VALUE IN DIFFERENTIAL DIAGNOSIS *

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A knowledge of the relative size of the red blood cells may be of definite value in differentiating between the types of anemia. This information may be obtained by a careful study of the red blood cells in a stained smear made from the patient's blood. Determinations of the mean diameter of the red blood cells by actual measurement of a number of cells, as suggested by Price-Jones,[1] or by a modification of this method, have been made use of frequently; but this method is rather too time-consuming to be used extensively. Pijper [2] and others have suggested a diffraction method for determining the size of the red blood cells. Haden [3] suggested the determination of the volume of the average red blood cell by means of a simple calculation based on a knowledge of the red blood cell count and the total percentage volume of cells as determined by means of the hematocrit. Jorgensen and Warburg [4] have reviewed much of the literature with reference to the size of the red blood cells in its relation to the anemias, so that a detailed discussion of this subject is not given here.

The observations here recorded were made with the idea of further determining the value of the average individual cell volume in a more or less routine study of a group of persons with normal blood and in a rather large group showing anemia. In some instances, measurements were made also of the mean diameter of the red blood cells.

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1. Price-Jones, C.: The Variation in the Sizes of the Red Blood Cells, Brit. M. J. **2**:1418, 1910.

2. Pijper, A.: An Improved Diffraction Method for Diagnosing and Following the Course of Pernicious and Other Anemias, Brit. M. J. **1**:635, 1929.

3. Haden, R. L.: Accurate Criteria for Differentiating Anemias, Arch. Int. Med. **31**:766 (May) 1923.

4. Jorgensen, S., and Warburg, E. J.: The Indices and Diameters of the Erythrocytes and the Best Haematological Criterion of Pernicious Anemia: I. Historical Notes and Normal Values, Acta med. Scandinav. **66**:109, 1926.

PROCEDURE

The procedure followed in these cases was essentially the same in each instance. With a dry syringe and needle, 10 cc. of blood was withdrawn from an arm vein and placed in a test tube previously prepared by evaporating to dryness 2 cc. of a 1.6 per cent solution of sodium oxalate as described by Keith, Rowntree and Geraghty [5] and by Murphy, Monroe and Fitz.[6] After the solution was mixed thoroughly by gently inverting the tube several times, standardized red blood cell-counting pipets were filled so that the red blood cells might be counted later. A determination of the hemoglobin by the Sahli method as modified by Osgood and Haskins [7] was made in many instances, although the determinations made by comparison with Tallqvist or Dare scales were used occasionally. Smears of the blood were made also so that the diameter of the red blood cells might be measured. In each instance 250 red blood cells were measured in stained smears of blood as described by Bell, Thomas and Means.[8] The oxalated blood was then centrifugated in a straight, thick-walled test tube at high speed (approximately 2,900 revolutions per minute) for one-half hour, and the percentage volume of the cells and plasma was determined after measuring the columns of each.

The average individual cell volume may be determined by dividing the percentage volume of red blood cells as determined by the hematocrit by the number of red blood cells in 100 cc. of oxalated venous blood. The figure is recorded in cubic centimeters times ten to the minus eleventh power. This calculation may be carried out according to the following simple formula in which 42 is the percentage volume of red blood cells as determined by the hematocrit and there are 5,000,000 red blood cells per cubic millimeter:

$$\frac{\text{Per cent of cells by hematocrit}}{\text{Number of R.B.C. in 100 cc. blood}} = \frac{42}{5{,}000{,}000 \times 1{,}000 \times 100} = 8.4 \times 10^{-11} \text{ cc.}$$

The cases so studied have been divided for convenience of discussion in the text into several groups. The observations recorded in each group are shown in the corresponding tables.

RED BLOOD CELLS IN NORMAL GROUP

Normal Persons.—Thirty-two determinations were made on thirty-one essentially normal persons, male and female, the ages varying from 20 to 70 years. In this group the average red blood cell count is 5,100,000 cells per cubic millimeter. The average percentage volume of red blood cells as determined by means of the hematocrit is 42.9. The average calculated individual cell volume is 8.42×10^{-11} cc.

5. Keith, N. M.; Rowntree, L. G., and Geraghty, J. T.: A Method for the Determination of Plasma and Blood Volume, Arch. Int. Med. **16**:547 (Oct.) 1915.

6. Murphy, W. P.; Monroe, R. T., and Fitz, R.: Changes in Composition of Blood in Pernicious Anemia: Treated by a Diet Rich in Liver, J. A. M. A. **88**:1211 (April 16) 1927.

7. Osgood, E. E., and Haskins, H. D.: A New Permanent Standard for Estimation of Hemoglobin by the Acid Hematin Method, J. Biol. Chem. **57**:107, 1923.

8. Bell, J. R.; Thomas, F. K., and Means, J. H.: Studies on Red Blood Cell Diameter: I. In Health and in Pernicious Anemia, J. Clin. Investigation **3**:229, 1926.

Normal Blood.—This group is made up of twenty-nine persons in whom no essential variation from normal could be demonstrated in the hemoglobin or red blood cell count in spite of the fact that they could not be considered well. Thirty-one determinations were made in this group. The average red blood cell count is 4,900,000 per cubic millimeter of blood. The average percentage volume of red blood cells is 40.8, whereas the average individual cell volume is 8.32×10^{-11} cc.

It may be seen from an analysis of tables 1 and 1A that the normal individual cell volume generally varies between 8×10^{-11} and 9×10^{-11}

TABLE 1.—*Observations on Normal Persons*

Case	Individual Cell Volume ($\times 10^{-11}$ Cc.)	Red Blood Cells in Millions per C.Mm.	Hematocrit, per Cent of Cells	Hemoglobin in Grams per 100 Cc.
1	9.1	4.94	45	13.76
2	9.0	4.80	43	15.04
3	9.0	4.79	43	13.76
4	8.9	5.30	47	13.76
5	8.9	5.28	47	14.50
6	8.9	4.92	44	13.76
7	8.7	4.58	40	11.73
8	8.6	4.87	42	12.14
9	8.6	4.78	41	13.76
10	8.6	5.14	44	14.62
11	8.6	4.99	43	12.55
12	8.6	5.24	45	13.76
13	8.5	4.83	41	14.62
14	8.5	4.85	41	13.76
15	8.5	5.19	44	13.52
16	8.5	5.54	47	12.90
17	8.4	5.00	42	13.76
18	8.4	4.88	41	14.62
19	8.3	4.60	38	12.01
20	8.3	5.19	43	14.62
21	8.3	5.51	46	13.22
22	8.2	5.45	45	15.59
23	8.2	4.75	39	11.59
24	8.1	4.93	40	11.04
25	8.1	5.56	46	14.90
26	8.1	5.21	42	12.29
27	8.0	5.74	46	13.76
28	8.0	5.62	45	13.76
29	8.0	5.37	43	14.50
29	7.9	5.30	42	13.76
30	7.9	4.84	38	11.19
31	7.8	5.11	40	13.22
Average	8.42	5.10	42.9	13.49

cc., although a small number, especially in the latter group, have an individual cell volume either greater than 9 or less than 8.

Haden's [3] average individual cell volume figure lies even above the upper limit considered as normal in this series. This is no doubt the result of two variations in our figures. Haden recorded an average red blood cell count which is lower than the one here recorded. On the other hand, the hematocrit percentage total cell volume figures recorded here are lower than those recorded by Haden, which suggests that the packing of the cells was greater in this series. Haden has suggested this possible variation, which is to be expected. The packing will vary according to the speed and duration of the centrifugation. In order to

make the individual cell volume figure useful, it will be necessary for each laboratory to establish a normal figure after centrifugating a small series of normal bloods. If the method is to be used infrequently it would be possible to centrifugate each time a tube of normal blood with the blood to be tested and use the normal individual cell volume figure so obtained as a control.

The percentage total red blood cell volume as determined by the hematocrit compares favorably with recent previously reported results,

TABLE 1A.—*Observations on Normal Blood*

Case	Individual Cell Volume ($\times 10^{-11}$ Cc.)	Red Blood Cells in Millions per C.Mm.	Hematocrit, per Cent of Cells	Hemoglobin in Grams per 100 Cc.	Diagnosis
1	9.4	5.00	47	15.48	Adenomatous goiter with hyperthyroidism
2	9.1	5.04	46	13.76	Cardiorenal disease
3	9.1	4.62	42	13.76	Hypertension; myocarditis
4	9.0	4.98	45	13.76	Myocarditis; slight uterine hemorrhage
5	8.9	4.70	42	12.28	Osteo-arthritis, chronic
6	8.9	4.61	41	13.76	Nutritional disturbance
7	8.9	4.60	41	12.90	Osteo-arthritis; mild hypothyroidism
8	8.8	4.67	41	12.14	Nutritional disturbance
9	8.8	4.30	38	11.73	Endometrial polyp
10	8.7	5.15	45	13.76	Sprue
11	8.7	4.95	43	14.35	Asthmatic bronchitis
12	8.7	4.62	40	13.11	Infectious jaundice, acute
12	8.5	4.47	38	12.00	Infectious jaundice, acute
13	8.6	4.64	40	12.90	Bronchopneumonia; asthma
14	8.4	4.62	39	12.90	Fibroid of uterus
15	8.4	4.40	37	13.76	Infectious jaundice, chronic
16	8.3	4.44	37	11.60	Question of duodenal ulcer
17	8.2	5.60	46	12.28	Infectious jaundice, acute
18	8.2	4.87	40	12.90	Nutritional disturbance
19	8.2	4.90	40	13.25	Duodenal ulcer; hypertension
19	8.0	4.50	36	11.88	Duodenal ulcer; hypertension
20	8.0	4.51	36	11.73	Hodgkin's disease
21	7.7	5.22	40	12.90	Nutritional disturbance
22	7.7	5.48	42	15.48	Mild hypothyroidism
23	7.7	4.95	38	11.73	Ulcerated colitis
24	7.7	4.73	36	12.15	Lacerated cervix; menopause
25	7.7	4.93	38	11.88	Intestinal indigestion
26	7.7	4.92	38	13.11	Obesity
27	7.6	5.82	44	12.97	Duodenal ulcer
28	7.4	6.05	45	12.83	Infarct of heart; coronary occlusion
29	7.1	5.90	42	13.93	Diabetes mellitus; jaundice
Average	8.32	4.90	40.8	13.00	

notably by Osgood,[9] Osgood and Haskins,[10] Jorgensen and Warburg[4] and Haden.[3]

Ponder[11] and others have determined the red blood cell volume from certain measurements of the cell. Harting[12] estimated the average

9. Osgood, E. E.: Hemoglobin, Color Index, Saturation Index and Volume Index Standards in Men, Arch. Int. Med. **37**:685 (May) 1926.

10. Osgood, E. E., and Haskins, H. D.: Relation Between Cell Count, Cell Volume and Hemoglobin Content of Venous Blood of Normal Young Women, Arch. Int. Med. **39**:643 (May) 1927.

11. Ponder, E.: The Erythrocyte and the Action of Simple Hemolysins, Edinburgh, Oliver & Boyd, 1924.

12. Harting: Das Mikroskop, Theorie, Gebrauch, Geschichte und gegenwärtiger Zustand desselben, Braunschweig, 1859, p. 950.

volume as 76 cubic microns. Welcker [13] obtained the figure of 72 cubic microns, whereas Ponder [11] has considered the average to be between 70 and 80 cubic microns. If the average individual cell volume figure 8.4×10^{-11} cc. obtained by us be changed to cubic microns, 84 cubic microns would represent the average normal red blood cell volume here observed. It is possible that this figure is slightly too high because, owing to the shape of the red blood cells, complete packing by means of the hematocrit method may not be possible.

RED BLOOD CELLS IN VARIOUS TYPES OF ANEMIA

Pernicious Anemia.—One hundred and thirty-two determinations were made on 106 patients with a diagnosis of pernicious anemia. The individual cell volume in the majority of instances was 10×10^{-11} cc. or greater. In general, this figure varied inversely with the red blood cell count, the lower figures being found in those cases with a more nearly normal count, and especially after treatment with liver or liver extract. In only a few of these cases was the red blood cell count definitely within normal limits, and very few determinations are recorded in this group, where patients had been treated with liver over a long period of time. Observations made on the blood of patients treated for longer periods of time and with a red blood cell count essentially normal are discussed in another paragraph. In a few of the cases studied complications were present. Only four cases (101, 102, 103, 104) recorded in table 2 need be discussed from this standpoint. In case 101 the patient had been treated with liver for about two years, during which time his red blood cell count was normal. He was then admitted to the hospital with an acute respiratory infection and acute nephritis, at which time this determination was made. Complete recovery occurred, and under liver treatment the patient has remained well for a year. Myxedema developed to complicate the picture in case 102. With thyroid extract and liver treatment, it has been possible for the patient to maintain a fair state of health, but with a red blood cell count generally somewhat below normal. The diagnosis in case 103 has been somewhat questionable. This patient is a negress who entered the hospital with a marked anemia. Sickle cells could not be demonstrated; free hydrochloric acid was absent from the gastric contents studied by the fractional test method for two hours after an Ewald test meal. Under treatment with liver, a reticulocyte rise occurred, followed by the usual red blood cell rise, and the patient has remained essentially well with similar treatment since. In case 104, the patient was started on liver treatment approximately two years before the recorded deter-

13. Welcker, H.: Grösse, Zahl, Volum Oberfläcke und Farbe der Blutkörperchen bei Menschen und bei Thieren, Ztschr. f. rat. Med. **20**:257, 1864.

TABLE 2.—*Observations in Pernicious Anemia*

Case	Individual Cell Volume ($\times 10^{-11}$ Cc.)	Red Blood Cells in Millions per C.Mm.	Hematocrit, per Cent of Cells	Case	Individual Cell Volume ($\times 10^{-11}$ Cc.)	Red Blood Cells in Millions per C.Mm.	Hematocrit, per Cent of Cells
1	17.6	0.91	16	48	11.8	1.69	20
2	17.4	0.57	10	49	11.8	2.46	48
3	16.8	1.01	17	49	10.7*	4.29	46
4	16.8	1.19	20	50	11.8	4.05	48
5	16.5	1.09	18	51	11.7	1.80	21
6	15.8	2.21	35	52	11.6	2.14	25
7	15.6	0.90	14	53	11.6	2.84	33
8	15.3	1.24	19	54	11.5	2.35	27
9	15.3	1.18	18	55	11.5	3.40	39
9	10.3*	2.42	25	56	11.4	1.40	16
9	9.4*	3.31	31	57	11.4	2.37	27
10	15.2	0.66	10	58	11.3	2.12	24
11	15.1	1.98	30	59	11.3*	3.38	38
11	11.6*	3.20	37	59	10.7*	3.56	38
12	14.8	1.35	20	59	8.6*	4.08	36
13	14.5	1.93	28	60	11.2	0.98	11
14	14.5	2.00	29	61	11.2	1.60	18
15	14.4	3.06	44	62	11.0	1.64	18
16	14.1	1.98	28	63	11.0	1.90	21
17	14.0	0.93	13	64	11.0	2.90	32
18	14.0	1.00	14	65	11.0	3.18	35
19	13.8	1.67	23	66	11.0	3.38	37
20	13.6*	1.99	27	67	10.9	3.13	34
20	10.5*	2.37	25	68	10.9*	3.30	36
20	13.3*	1.88	25	69	10.8*	5.20	56
20	13.1*	1.98	26	70	10.7*	3.94	42
21	13.5	1.70	23	71	10.7*	4.20	45
22	13.5	1.70	23	72	10.6	1.42	15
22	10.9*	3.20	35	72	9.7*	3.08	30
23	13.5	3.19	43	73	10.6	1.79	19
23	11.6	2.52	29	74	10.6	2.07	22
24	13.3	1.05	14	75	10.6	2.73	29
25	13.3	1.35	18	76	10.6	3.30	35
26	13.1	1.53	20	76	10.4	3.56	37
26	10.2*	4.00	41	77	10.5*	4.10	43
27	13.1	4.20	55	78	10.4	2.30	24
28	13.0	2.74	36	79	10.4*	3.76	39
28	10.0*	3.90	39	80	10.4*	4.24	44
29	13.0	3.15	41	81	10.3	2.13	22
29	9.1*	4.18	38	82	10.3	2.90	30
30	12.8	1.80	23	83	10.2	3.51	36
30	9.4	1.70	16	84	10.2*	4.00	41
30	11.6	1.64	19	85	10.2*	4.12	42
30	11.4	1.74	20	86	10.1	3.44	35
30	14.0	1.78	25	87	10.1*	3.45	35
31	12.8*	3.10	40	88	10.1	3.97	40
32	12.7	1.10	14	89	10.0	1.40	14
32	12.6	1.03	13	90	10.0	1.50	15
33	12.7	1.18	15	90	9.7*	1.45	14
34	12.7	1.42	18	90	9.9*	3.63	35
35	12.7	2.60	33	91	10.0	1.80	18
36	12.7	2.76	35	92	10.0	3.10	31
37	12.7	3.30	42	96	10.0*	3.50	35
38	12.4	2.90	36	94	10.0*	4.70	47
38	9.0*	4.24	38	95	9.8*	4.50	44
39	12.3	2.20	27	96	9.7*	3.30	32
39	10.5*	2.76	29	97	9.6*	3.98	38
40	12.3	3.00	37	98	9.1*	4.29	39
41	12.3	3.64	45	99	9.1*	4.50	41
42	12.2	1.64	20	100	8.9*	3.84	34
43	11.9	1.51	18	101	8.9*	4.50	40
44	11.9	1.60	19	102	8.8*	3.54	31
44	13.0	1.69	22	103	8.1	1.60	13
45	11.8	1.02	12	104	7.4	3.53	26
46	11.8	1.10	13	105	7.4	3.90	29
47	11.8	1.52	18	106	6.9	4.66	32

* Some liver was ingested before observation was made.

minations were made. At that time the history, symptoms, physical signs and blood examination were all typical of pernicious anemia with changes resulting from an early combined degeneration of the spinal cord. At the time of the observation here recorded there was a drop in the blood count; occult blood was demonstrated in the feces, and there was evidence of progression of the cord changes. Roentgen examination of the stomach showed a lesion, probably carcinoma. Exploratory operation was refused. The diagnosis of pernicious anemia, although recorded, is questionable in cases 105 and 106. In each instance no free hydrochloric acid was found in the gastric contents. The color index, smear examination and history, however, suggest a secondary type of anemia. Hemorrhage from the intestinal tract was demonstrated quite certainly in case 105, although roentgenologic examination failed to demonstrate a pathologic condition.

Questionable Pernicious Anemia.—Five other cases with high individual cell volume figures recorded in table 2A might properly be discussed at this point. The first three cases are placed in this group because it was impossible to make a positive diagnosis of pernicious anemia, although certain features of the cases were suggestive of this disease. In each instance free hydrochloric acid was absent from the gastric contents, and the blood picture would fit in satisfactorily for this diagnosis, although there were certain atypical symptoms. In case 2, the patient was admitted with symptoms suggesting disease of the gallbladder, but cholecystographic studies failed to show such a disturbance. In case 3, there was a basal metabolic rate of +36 per cent and an atypical anemia. The diagnosis was recorded in the hospital record as chronic alcoholism, Korsakoff's syndrome. Case 4 [14] showed an absence of free hydrochloric acid in the gastric contents and definite macrocytosis in the blood smear. There was, however, occult blood in the feces, and roentgenologic studies showed a large mass in the stomach. Liver extract was given, followed by a rise in the reticulocyte count and in the red blood cell count. An exploratory operation revealed an adenocarcinoma involving a large part of the posterior wall of the stomach. Although a part of the anemia was the result of loss of blood, it is quite likely that pernicious anemia was also present. The diagnosis must remain somewhat in doubt in case 5 also. This patient had a moderate degree of anemia, not typical of that found with pernicious anemia and a small amount of free hydrochloric acid was found in the gastric contents. Symptoms were present, however, suggesting rather extensive disturbance in the central nervous system, not entirely typical

14. This patient was observed through the courtesy of Dr. J. P. O'Hare and Dr. John Homans of the Peter Bent Brigham Hospital staff.

of those found in combined systemic disease involving the posterior and lateral columns of the cord. A moderate increase in the red blood cell count resulted from treatment with liver. A diagnosis of multiple sclerosis was made.

Anemia Due to Chronic Loss of Blood.—The record of individual cell volume determinations in fifty-two patients having anemia resulting from chronic loss of blood are recorded in table 3. In the majority of these cases many determinations were made; but in each instance the first determination is recorded only when the anemia was most marked. The individual cell volume figure for this group of cases varies from the very low one of 5.2×10^{-11} cc. to the average normal figure of 8.5×10^{-11} cc. Only about 20 per cent of the figures fall within the range which is considered normal. In no case here recorded was the figure higher than normal, which was found in the cases of pernicious

TABLE 2A.—*Observations in Questionable Pernicious Anemia*

Case	Individual Cell Volume ($\times 10^{-11}$ Cc.)	Red Blood Cells in Millions per C.Mm.	Hematocrit, per Cent of Cells
1	9.9	2.72	27
1	10.5	2.68	28
2	11.6	3.10	36
2	11.0	3.17	35
2	10.6	3.57	38
3	10.9	3.57	39
4	11.5	1.83	21
4	10.5	2.10	22
5	10.0	4.10	41

anemia. It is interesting to note, however, that during improvement of the blood condition under the influence of treatment the individual cell volume, when low at first, gradually increased to a normal figure. Figures illustrating this point are shown in table 3A (case 3 of table 3). The cause of the loss of blood appears not to have been a factor in determining the size of the cells. Likewise, there seems to be no definite relation between the cell volume and the red blood cell or hemoglobin level before treatment was started.

Anemia Due to Acute Loss of Blood.—In table 4 are recorded twenty-eight observations made in twelve patients having hemorrhage either just prior to or during the period of observation. In these patients the average individual cell volume was greater than the normal cell volume in most instances shortly following the hemorrhage. The observations recorded in cases 1 and 4 show well the gradual change to normal or even smaller than normal individual cell volume during improvement in the blood. No special treatment was given in these two cases to enhance blood formation. In case 12, bleeding occurred

TABLE 3.—*Observations in Anemia Due to Chronic Loss of Blood*

Case	Individual Cell Volume ($\times 10^{-13}$ Cc.)	Red Blood Cells in Millions per C.Mm.	Hematocrit, per Cent of Cells	Hemoglobin in Grams per 100 Cc.	Source of Hemorrhage
1	5.2	4.80	25	6.88	Cancer of ascending colon
2	5.3	5.10	27	6.62	Uterine hemorrhage
3	5.6	4.85	27	5.94	Uterine hemorrhage
4	5.6	3.76	22	5.50	Uterine hemorrhage
5	5.7	4.00	23	4.14	Hemorrhoids
6	5.8	4.80	28	8.55	Uterine hemorrhage
7	6.0	2.17	13	2.34	Uterine hemorrhage
8	6.1	5.40	33	8.00	Uterine hemorrhage
9	6.2	3.57	22	4.28	Uterine hemorrhage
10	6.2	3.40	21	4.83	Uterine hemorrhage
11	6.3	4.30	27	10.03	Intestinal hemorrhage
12	6.3	5.10	32	7.17	Uterine hemorrhage
13	6.4	4.71	30	7.58	Uterine hemorrhage
14	6.6	4.09	27	6.62	Uterine hemorrhage
15	6.6	3.97	26	7.17	Uterine hemorrhage
16	6.7	4.60	31	7.58	Uterine hemorrhage
17	6.7	2.97	20	3.45	Hemorrhoids
18	6.8	3.10	21	7.74	Cancer of stomach
19	6.8	3.38	23	4.55	Uterine hemorrhage
20	6.8	4.71	32	9.65	Intestinal hemorrhage
21	6.9	2.17	15	6.88	Cancer of stomach
22	6.9	2.75	19	5.16	Cancer of stomach
23	7.0	3.72	26	6.02	Cancer of stomach
24	7.1	3.40	24	4.96	Uterine hemorrhage
25	7.1	2.97	21	3.17	Hemorrhoids
26	7.2	2.35	17	2.07	Cancer of intestine
27	7.2	4.06	29	5.79	Uterine hemorrhage
28	7.2	4.74	34	8.70	Uterine hemorrhage
29	7.3	4.55	33	11.19	Uterine hemorrhage
30	7.4	1.85	14	4.14	Uterine hemorrhage
31	7.4	2.73	20	5.52	Uterine hemorrhage
32	7.5	3.20	24	6.88	Hemorrhoids
33	7.5	4.30	32	10.03	Uterine hemorrhage
34	7.5	3.10	23	6.21	Uterine hemorrhage
35	7.5	4.69	35	10.03	Uterine hemorrhage
36	7.6	3.02	23	4.97	Uterine hemorrhage
37	7.6	3.82	29	6.06	Uterine hemorrhage
38	7.7	3.14	24	4.42	Uterine hemorrhage
39	7.7	3.49	27	5.38	Uterine hemorrhage
40	7.8	2.70	21	4.42	Uterine hemorrhage
41	7.8	3.00	23	3.31	Uterine hemorrhage
42	7.9	2.80	22	6.88	Cancer of stomach
43	7.9	3.30	26	7.31	Uterine hemorrhage
44	8.1	4.31	35	9.80	Uterine hemorrhage
45	8.2	3.66	30	8.28	Uterine hemorrhage
46	8.2	3.68	30	8.55	Hemorrhoids
47	8.3	1.80	15	3.45	Hemorrhoids
48	8.3	2.64	22	4.68	Intestinal hemorrhage
49	8.3	3.00	25	6.21	Hemorrhoids
50	8.4	2.75	23	4.14	Cancer of stomach
51	8.5	2.71	23	8.60	Hemorrhoids
52	8.5	3.64	31	8.60	Hemorrhoids

TABLE 3A.—*Increase in Individual Cell Volume (Case 3, Table 3)*

Date of Test	Individual Cell Volume ($\times 10^{-11}$ Cc.)	Red Blood Cells in Millions per C.Mm.	Hematocrit, per Cent of Cells	Hemoglobin in Grams per 100 Cc.
11/13/28	5.6	4.85	27	5.94
11/19/28	6.3	4.27	27	5.52
11/26/28	5.9	4.77	28	6.06
12/ 2/29	5.4	4.97	27	5.79
12/12/28	5.3	4.89	26	6.48
12/19/28	5.7	4.54	26	5.94
12/26/28	5.2	4.99	26	6.06
1/ 7/29	6.5	4.90	32	6.48
1/14/29	7.1	5.19	37	9.10
1/23/29	8.2	4.87	40	10.08
2/13/29	8.6	5.24	45	12.99

frequently during the period of observation, which no doubt explains the rather constantly increased cell volume. Cases of this series would hardly be confused clinically with cases of pernicious anemia as the presence of hemorrhage was very obvious.

"Secondary" Anemia Not Caused by Hemorrhage.—In a group of twenty-four patients having a secondary anemia from some cause other than hemorrhage, the cell volume varied considerably, about one-third being below normal, whereas a smaller group showed greater than

TABLE 4.—*Observations in Anemia Due to Acute Loss of Blood*

Case	Date of Test	Individual Cell Volume ($\times 10^{-11}$ Cc.)	Red Blood Cells in Millions per C.Mm.	Hematocrit, per Cent of Cells	Hemoglobin in Grams per 100 Cc.	Source of Hemorrhage
1	10/ 1	11.3	2.17	25	6.75	Gastric ulcer
1	10/11	9.3	2.70	25	5.80	
1	10/19	8.6	3.00	26	6.48	
1	12/ 2	7.0	4.70	33	7.86	
2	.....	10.5	2.10	22	6.21	Postoperative
3	1/12	10.1	2.68	27	8.14	Gastric ulcer
3	2/ 2	8.0	3.23	26	12.05	
4	10/21	10.1	1.97	20	4.42	Gastric ulcer
4	10/27	8.7	2.88	25	5.80	
4	11/ 5	8.2	3.55	29	6.75	
4	11/11	8.4	3.70	31	6.75	
4	11/18	7.5	4.54	34	9.38	
4	12/ 2	6.5	4.76	31	8.41	
4	12/16	7.3	5.10	37	10.05	
5	.....	9.9	2.71	27	7.17	Uterine hemorrhage
6	.....	9.9	3.14	31	8.70	Uterine hemorrhage
7	.....	9.8	2.76	27	6.75	Uterine hemorrhage
8	.....	9.3	3.54	33	11.03	Hemorrhoids
9	.....	9.2	1.74	16	2.90	Uterine hemorrhage
10	.....	8.7	2.87	25	6.75	Gastric ulcer
11	.....	7.4	3.36	25	7.59	Gastric ulcer
12	1/ 5	7.4	2.16	16	4.14	Hemorrhoids; epistaxis
12	1/18	11.7	2.40	28	6.35	
12	1/26	9.7	2.90	28	7.17	
12	2/ 3	9.9	3.13	31	9.52	
12	2/10	10.2	3.53	36	9.90	
12	2/17	9.5	3.88	37	11.60	
12	2/24	9.3	4.09	38	11.47	

normal volume. The anemia in some of these cases was probably either of the chronic chlorotic type or to be explained on the basis of nutritional disturbances. In these cases the diagnosis is recorded as "undetermined." With the exception of the last patient, the individual cell volume is definitely smaller than that usually found during relapse in patients with pernicious anemia. The cause for the anemia in this last case was undetermined. The patient's mother had pernicious anemia, but the patient herself had free hydrochloric acid in the gastric contents and no symptoms suggestive of this disease.

Pregnancy with Anemia.—The individual cell volume was studied in six patients having an anemia during pregnancy or following delivery.

TABLE 5.—*Observations in "Secondary" Anemia Not Caused by Hemorrhage*

Case	Individual Cell Volume ($\times 10^{-12}$ Cc.)	Red Blood Cells in Millions per C.Mm.	Hematocrit, per Cent of Cells	Hemoglobin in Grams per 100 Cc.	Diagnosis
1	6.5	3.98	26	6.88	Nutritional disease
1	9.8	4.50	44	13.40	(After treatment with liver)
2	6.7	3.76	25	5.94	Diaphragmatic hernia
2	7.7	5.31	41	12.55	(After treatment with liver)
3	6.9	4.05	28	8.60	Nutritional disease
4	7.0	5.72	40	10.03	Chronic chlorosis
5	7.2	4.16	30	10.01	Erythema nodosum
6	7.5	4.69	35	10.03	Chronic chlorosis
7	7.5	4.65	35	8.60	Chronic chlorosis
8	7.9	4.55	36	9.37	Myxedema
9	7.9	4.21	33	10.05	Meningioma
10	8.1	2.46	20	7.74	Salpingitis; chronic oophoritis
11	8.3	3.40	28	8.27	Subacute bacterial endocarditis
12	8.3	4.32	36	....	Cholecystitis; cholelithiasis
12	8.4	3.10	26	7.86	
13	8.3	4.21	35	11.04	Myocarditis
13	8.9	3.14	28	8.82	
14	8.4	4.67	39	11.19	Not determined
15	8.5	3.61	31	8.69	Lead poisoning, chronic
16	8.6	5.10	44	11.19	Osteo-arthritis
17	8.6	4.06	35	10.04	Question of methemoglobinemia (paroxysmal)
18	8.7	4.94	43	12.03	Arteriosclerosis
19	8.7	4.37	38	12.03	Mild hypothyroidism
20	8.7	2.65	23	6.20	Obesity; nutritional disease
21	8.7	1.83	16	2.34	Chronic myocarditis; cerebral arteriosclerosis
22	8.8	4.32	38	12.03	Not determined
23	8.9	3.61	32	7.60	Duodenal ulcer; arteriosclerosis peripheral
24	8.9	3.92	35	10.03	Arthritis, infectious
24	9.2	4.68	43	12.90	(After treatment with liver)
24	8.0	4.25	34	9.90	(After treatment with liver)
25	9.0	5.02	45	11.19	Not determined
26	9.0	4.99	45	10.03	Chronic chlorosis
27	9.0	3.33	30	9.40	Myxedema
28	9.2	5.10	47	12.03	Not determined
29	9.3	3.78	35	10.05	Hemopneumothorax; massive collapse of lung
30	9.3	3.00	28	7.60	Carcinoma of head of pancreas; jaundice
31	9.3	4.28	40	12.03	Not determined
32	10.0	4.08	41	11.19	Not determined

TABLE 6.—*Observations in Pregnancy with Anemia*

Case	Individual Cell Volume ($\times 10^{-12}$ Cc.)	Red Blood Cells in Millions per C.Mm.	Hematocrit, per Cent of Cells	Hemoglobin in Grams per 100 Cc.	Comment
1	11.5	1.22	14	2.48	Postpartum anemia
1	9.0	4.24	38	11.19	One year later free hydrochloric acid present
2	9.7	3.90	38	12.90	During pregnancy
3	8.9	3.40	30	8.96	During pregnancy
4	8.8	2.44	21	5.10	Postpartum anemia
4	8.3	4.20	35	10.01	
4	8.2	4.40	36	11.45	
4	9.2	4.43	41	11.45	
4	8.6	4.73	41	11.18	
4	7.4	5.40	40	11.45	
5	7.5	2.94	22	6.06	During pregnancy
5	6.8	4.42	30	8.00	
5	6.9	4.80	33	7.72	
5	7.1	4.80	34	8.42	
5	7.6	4.76	36	8.82	
5	8.0	4.96	39	10.06	
5	8.6	4.43	38	10.02	
5	8.5	4.73	40	11.04	
6	6.3	4.76	30	5.52	During pregnancy; no free hydrochloric acid present

As may be seen from table 6, the figure for the individual cell volume varied considerably. In cases 1 and 4, the anemia occurred acutely after delivery and was not due to hemorrhage. Whether or not slight anemia had occurred previously in these patients is not known. Free hydrochloric acid was found in the gastric contents of the first patient. Only one other patient (case 6) was tested in this manner, and no free hydrochloric acid was found in specimens obtained up to two hours following an Ewald test meal. The anemia in this patient and in the others of the group, excepting in cases 1 and 4, was observed during the course of pregnancy. In each patient of the series the blood picture in most respects was characteristic of the secondary type. Although the cell

TABLE 7.—*Observations in Nephritis*

Case	Individual Cell Volume ($\times 10^{-12}$ Cc.)	Edema	Red Blood Cells in Millions per C.Mm.	Hematocrit, per Cent of Cells	Hemoglobin in Grams per 100 Cc.	Diagnosis
1	8.2	..	3.42	28	7.86	Chronic nephritis without edema
2	10.0	++++	2.70	28	8.55	Chronic nephritis with edema
2	9.2	++++	3.36	31	8.42	
2	8.4	+++	3.65	31	9.24	
2	8.0	++	3.15	25	8.27	
2	7.8	+	3.30	26	8.00	
2	9.6	+	2.90	28	6.76	
2	8.7	+	3.31	29	8.00	
2	9.0	+	3.75	34	10.05	
3	9.1	++++	3.74	34	10.06	Chronic nephritis with edema
3	7.5	++	5.03	38	10.08	
3	8.2	+++	3.90	32	10.05	
3	8.4	++	3.80	32	10.02	
3	8.6	++	3.58	31	10.02	
3	7.0	+	3.85	27	10.02	
3	8.2	+	3.56	29	8.55	
3	10.0	+++	3.82	38	9.38	
3	8.6	++	3.60	31	8.96	
4	10.2	..	1.57	16	7.74	Chronic nephritis without edema
5	11.4	++++	4.38	49	12.90	Chronic nephritis with edema

volume was definitely above the normal range in two cases of this series (cases 1 and 2), it is not likely that any of them fall into the group of so-called pernicious anemia of pregnancy.

Anemia in Nephritis.—Data concerning five patients with nephritis are given in table 7. Here again the individual cell volume in several instances was greater than the upper normal figures. The reason for this is not easy to determine, although clinically there was no difficulty in distinguishing them from the pernicious anemia group. Liver therapy failed to increase the red blood cells. Three patients of the group had nephritis with edema and the cases may be classed as so-called nephrosis. The remaining two patients had nephritis without edema. In those patients with edema, the individual cell volume was apparently influenced by the presence or absence of the edema, usually being increased during the periods of increased fluid retention.

RED BLOOD CELLS IN ERYTHREMIA AND MISCELLANEOUS BLOOD DISEASES

Erythremia.—Five patients with erythremia were observed, and the results are shown in table 8. In these patients, the individual cell volume was within or below the normal range.

TABLE 8.—*Observations with Erythremia*

Case	Individual Cell Volume ($\times 10^{-11}$ Cc.)	Red Blood Cells in Millions per C.Mm.	Hematocrit, per Cent of Cells	Hemoglobin in Grams per 100 Cc.	Diagnosis
1	7.1	6.10	43	13.8	Erythremia; cerebral thrombosis
1	9.1	5.82	53	13.8	
2	7.2	8.19	59	16.44	Erythremia
3	7.4	6.06	45	17.20	Erythremia
4	8.0	8.90	71	17.27	Erythremia
4	9.5	7.30	69	15.05	
5	8.6	6.80	59	15.47	Erythremia; hypertension

TABLE 9.—*Observations in Miscellaneous Blood Diseases*

Case	Individual Cell Volume ($\times 10^{-11}$ Cc.)	Red Blood Cells in Millions per C.Mm.	Hematocrit, per Cent of Cells	Hemoglobin in Grams per 100 Cc.	White Blood Cells per C.Mm.	Diagnosis
1	15.2	1.95	30	6.06	3,500	Leukemia, aleukemic lymphoid
2	12.8	2.10	27	7.59	2,900	Leukemia, aleukemic lymphoid
3	11.6	1.81	21	5.11	2,700	Leukemia, aleukemic lymphoid
4	12.1	1.48	18	....	.....	Malignant lymphoma; jaundice
5	11.6	1.80	21	5.25	20,000	Leukemia, chronic myeloid
6	11.1	1.35	15	2.90	3,000	Purpura hemorrhagica, acute
7	11.1	1.35	15	2.34	10,000	Leukemia, aleukemic myeloid
8	11.1	1.26	14	2.07	8,600	Leukemia, aleukemic myeloid
9	10.2	1.95	20	4.84	11,000	Leukemia, aleukemic myeloid
10	10.0	1.70	17	5.16	41,000	Leukemia, chronic lymphatic
11	10.0	1.60	16	5.16	21,500	Leukemia, chronic myeloid
12	9.9	0.91	9	1.52	2,100	Atypical blood disease
13	9.7	1.86	18	7.74	.....	Leukemia, aleukemic myeloid
14	9.5	2.73	26	7.18	2,700	Atypical blood disease
15	9.4	4.69	44	12.02	204,100	Leukemia, chronic myeloid
15	8.1	4.69	38	12.02	204,100	Leukemia, chronic myeloid
16	9.3	2.15	20	4.84	5,200	Leukemia, aleukemic myeloid
16	9.3	1.81	17	3.86	5,500	Leukemia, aleukemic myeloid
17	9.2	2.50	23	9.45	12,000	Atypical blood disease
18	8.9	4.90	44	5.25	190,000	Leukemia, chronic myeloid
18	6.5	4.90	32	5.25	190,000	Leukemia, chronic myeloid
19	8.0	2.50	20	4.14	90,000	Leukemia, chronic myeloid
20	7.7	3.65	28	9.45	3,800	Leukemia, aleukemic myeloid
21	6.5	4.60	30	9.45	6,000	Splenomegaly

Miscellaneous Blood Diseases.—In table 9 are recorded observations on a group of patients having some form of essential blood disturbance other than pernicious anemia. In many of these cases, especially those of aleukemic leukemia, it was difficult to distinguish from pernicious anemia. The determination of the individual cell volume did not help in this differentiation. The table shows that the majority of the determinations fall within the range generally found during relapse in patients with pernicious anemia. In only two patients (cases 10 and 12) was it considered necessary to subtract the height of the white blood cell column

from the total cell column in determining the cell hematocrit figure, although it is possible that a somewhat lower individual cell volume would have been found in other cases if this had been done. Difficulties in diagnosis were slight in those cases having the higher white blood cell counts, although diagnosis was difficult in the cases having low white blood counts. It is in such cases as the latter that aid in diagnosis is desired, but differentiation is not possible by a determination of the individual cell volume. A noteworthy fact is that the individual cell volume was normal or less than normal in the patients with the more nearly normal red blood cell counts. Such a state of affairs did not occur in the patients with pernicious anemia with higher counts, even if liver had been ingested for short periods of time.

COMPARISON WITH MEASUREMENTS OF THE MEAN DIAMETER

Measurements of the average individual cell volume and of the mean cell diameter were made on the same sample of venous blood in a series of patients with pernicious anemia and in a group of patients with various diagnoses. The mean diameter of the red blood cells in normal persons has been found by Bell, Thomas and Means [8] to vary from 7.4 to 8 microns and by Medearis and Minot [15] from 7.25 to 7.75 microns. These figures compare favorably with those obtained by others and especially those collected from the literature by Jorgensen and Warburg [4] and by Ponder.[11] The mean diameter rarely exceeds 7.8 microns and for the purposes of this paper 8 microns is considered the upper limit of normal. Twenty-seven determinations were made of both the individual cell volume and the mean diameter of the red blood cells of twenty-five patients with pernicious anemia, in whom treatment with liver had been carried out for only brief periods of time or not at all, or in whom the red blood cell count had dropped because an insufficient amount of liver had been taken. In two of the patients the observations were made on two separate specimens of blood. The data is presented for comparison in table 10. The cases have been arranged according to the size of the mean cell diameter. In the first five cases, the mean diameter was within the normal range. In none of these five patients was the individual cell volume normal. In fact, in only two cases of the entire series was the individual cell volume figure within the normal range, namely, case 18 (case 102, table 2) and case 20 (case 103, table 2). The mean diameter measurement in both of these cases was distinctly greater than normal. It is very likely that in case 18 the individual cell volume figure represents the condition of the blood more

15. Medearis, D. N., and Minot, G. R.: Studies on Red Blood Cell Diameter: II. In Pernicious Anemia, Before and During Marked Remission, and in Myelogenous Leukemia, J. Clin. Investigation **3**:541, 1927.

nearly than the mean diameter figure, as this patient had developed signs and symptoms of myxedema with a basal metabolic rate of —29 per cent. A more detailed discussion of this case has been recorded under case 102 of table 2. Case 20 has been discussed previously as case 103

TABLE 10.—*Comparison of Mean Diameter with Individual Cell Volume in Pernicious Anemia*

Case	Mean Diameter, Microns	Individual Cell Volume ($\times 10^{-11}$ Cc.)	Red Blood Cells in Millions per C.Mm.	Hemoglobin in Grams per 100 Cc.
1	7.59	10.5	4.09	11.88
2	7.80	11.8	1.52	5.79
3	7.90	11.7	1.79	8.77
4	7.94	13.8	1.67	8.60
5	7.99	11.8	1.69	7.45
6	8.01	11.0	2.90	11.20
7	8.02	16.8	1.05	11.18
8	8.07	9.9	2.72	7.86
9	8.13	12.7	1.42	4.97
9A*	8.75	10.6	3.59	11.30
10	8.15	11.3	2.03	4.83
11	8.17	11.0	3.18	10.35
12	8.19	15.6	0.90	5.16
13	8.22	16.1	1.02	7.74
13A†	8.46	10.0	1.80	5.24
14	8.24	10.2	3.51	10.49
15	8.33	15.8	2.21	8.00
16	8.39	11.8	1.01	3.45
17	8.46	12.7	1.15	3.04
18	8.48	8.8	3.54	11.60
19	8.65	11.9	1.60	6.02
20	8.75	7.9	4.03	9.38
21	8.80	14.8	1.35	....
22	8.86	12.7	3.31	11.02
23	8.93	12.8	3.12	9.65
24	8.94	11.9	2.94	6.21
25	9.10	10.2	2.54	8.83

* 9A one year later than determination no. 9.
† 13A two weeks later than determination no. 13.

TABLE 11.—*Comparison of Mean Diameter with Individual Cell Volume in Cases of Pernicious Anemia in Which Treatment Has Been Given*

Case	Mean Diameter, Microns	Individual Cell Volume ($\times 10^{-11}$ Cc.)	Red Blood Cells in Millions per C.Mm.	Hematocrit, per Cent of Cells	Hemoglobin in Grams per 100 Cc.
1	7.49	8.4	4.98	42	12.98
2	7.60	8.2	4.91	40	11.30
3	7.70	8.7	5.51	48	13.90
4	7.70	8.7	4.92	43	12.29
5	7.90	8.7	4.59	40	12.29
6	7.98	8.9	4.50	40	11.02
6	8.70	9.0	4.90	44	12.84
7	8.10	8.4	5.23	44	12.84
8	8.10	9.3	4.50	42	11.02
9	8.30	9.1	4.52	41	12.14
10	8.30	9.1	4.96	45	13.25
11	8.50	9.4	4.98	47	13.10
12	8.60	9.2	4.22	39	11.31

of table 2. In this group of patients the individual cell volume figure gave more definite information concerning the state of the red blood cells than did the much more difficult and technical determination of the mean cell diameter.

Studies were made on twelve patients with pernicious anemia who had received satisfactory liver treatment for a year or more during which time their red blood cell count had remained at or above 4,500,000 per cubic millimeter except in the patient recorded in case 12. The count in this case had remained above this level up to the time the cell diameters were measured when it was 4,220,000 per cubic millimeter. In one patient observations were made on two occasions about seven months apart so that a total of thirteen observations are recorded. In six instances both the mean diameter and individual cell volume figures fell within the normal range, whereas in two others, although the individual cell volume was normal, the mean diameter was greater than normal. In no instance, however, was the individual cell volume higher than the upper limit reached in a few of the normal blood specimens as recorded in tables 1 and 1A.

The figures noted in table 11 suggest that under proper liver treatment the red blood cells in a patient with pernicious anemia may be maintained at an essentially normal volume with, in some instances, a slightly greater than normal mean cell diameter. These observations corroborate the figures reported by Bell, Thomas and Means [8]; Medearis and Minot,[15] Persons,[16] and Fitzhugh and Persons,[17] obtained by measurement of the mean diameter of the red blood cells in a group of patients with pernicious anemia treated with liver.

In the third group of cases in which a comparison was made of the individual cell volume and the mean cell diameter, thirty observations were made on twenty-four patients and the data are given in table 12. A detailed discussion of the figures will not be undertaken, as the points of difference in the two determinations may be seen readily by inspection of the table. Mention is made, however, of some aspects of those cases in which marked difference between the two figures occur. In case 1 (also case 1 of table 6) the patient had severe, acute anemia following labor. Hemorrhage did not occur. One year later, the individual cell volume was 9×10^{-11} cc. when the red blood cell count was only slightly below normal and the patient apparently in good health. At this time free hydrochloric acid was found in the gastric contents following an Ewald test meal. In recording the individual cell volume of case 13, no allowance was made for the increase of white blood cells which no

16. Persons, E. L.: Studies on Red Blood Cell Diameter: III. The Relation of the Diameter of Immature (Reticulocytes) and Adult Red Blood Cells in Health and Anemia, Especially in Pernicious Anemia, J. Clin. Investigation **7**: 615, 1929.

17. Fitzhugh, G., and Persons, E. L.: Studies on Red Blood Cell Diameter: IV. The Decrease in the Mean Diameter of the Reticulocytes and Adult Red Blood Cells in Pernicious Anemia Following Liver Therapy, J. Clin. Investigation **7**:631, 1929.

doubt influenced the figure. This case, as well as cases 15 and 23 and 23A, are recorded in table 9. Attention was called previously to the fact that many of this group of cases showed a high cell volume figure. Only in cases 23, 23A do the two figures agree as to the cell size. More determinations must be made in order to decide definitely whether or not there is actually an increase in red cell size in such cases.

In three cases (16, 17 and 24, table 12) the anemia was the result of acute loss of blood. Observations of both the individual cell volume

TABLE 12.—*Comparison of Mean Diameter with Individual Cell Volume in Miscellaneous Cases*

Case	Mean Diameter, Microns	Individual Cell Volume ($\times 10^{-11}$ Cc.)	Red Blood Cells in Millions Per C.Mm.	Hemoglobin in Grams per 100 Cc.	Diagnosis
1	6.47	11.5	1.22	2.48	Pregnancy and anemia
2	6.88	8.4	2.75	4.14	Carcinoma of stomach
3	7.00	6.7	3.76	5.94	Diaphragmatic hernia
4	7.12	8.1	5.56	14.90	No disease
5	7.13	7.0	5.72	12.90	Achylia gastrica
6	7.15	8.3	4.32	13.77	Gallstones; jaundice
7	7.16	8.2	3.40	8.28	Subacute bacterial endocarditis
8	7.18	9.3	4.28	12.02	Nutritional anemia
9	7.21	9.3	3.54	11.02	Arteriosclerosis
10	7.22	8.5	4.47	12.00	Gallstones; cirrhosis; jaundice
11	7.24	7.2	4.16	10.08	Erythema, nodosum
12	7.29	7.1	3.41	4.96	Fibroma of uterus
12A	7.35	7.0	3.31	4.83	Fibroma of uterus
13	7.34	10.0	1.70	5.16	Lymphatic leukemia
14	7.36	6.8	4.71	12.02	Carcinoma of colon
15	7.36	10.2	1.95	4.83	Leukemia, aleukemic myeloid
16	7.38	8.0	3.23	12.02	Duodenal ulcer
16A	7.73	10.1	2.68	8.14	Duodenal ulcer; acute hemorrhage
17	7.42	7.4	2.16	4.14	Hemorrhoids; acute hemorrhage; epistaxis
17A	7.59	9.9	3.13	8.28	Hemorrhoids; acute hemorrhage
17B	7.83	10.2	3.53	9.94	Hemorrhoids; acute hemorrhage
18	7.40	8.3	4.21	11.02	Myocarditis
19	7.68	7.0	4.70	7.86	Ulcer with hemorrhage
20	7.68	8.3	4.44	11.60	Ulcer with hemorrhage (question)
21	7.70	6.5	4.76	8.41	Ulcer with hemorrhage
21A	7.70	7.3	5.10	10.50	Ulcer with hemorrhage
22	7.76	8.0	4.25	9.94	No disease
23	7.98	9.3	1.81	3.86	Leukemia, aleukemic myeloid
23A	8.04	9.3	2.15	4.84	Leukemia, aleukemic myeloid
24	8.27	10.5	2.13	9.46	Carcinoma of stomach

and the mean cell diameter measurement made shortly following or during the period of loss of blood showed an increase in the size of the red blood cells. The figures obtained at this time are shown under 16A, 17B and 24 (table 12). Later, after the blood loss had ceased and the count was more nearly normal, the size was found to be essentially normal by both methods, as shown under 16 and 17 (table 12). In one observation (17A), the figures do not agree as to the size of the cells, the individual cell volume being larger than normal, whereas the mean diameter is within normal limits. This patient lost blood from time to time during the period of observation and repeated individual cell volume studies showed a figure greater than the normal range. This case is also listed in table 4 as case 12. As was mentioned

in the discussion of this table, the individual cell volume figure was larger than normal in most instances shortly after hemorrhage.

From an analysis of the figures in table 12, the evidence suggests that the individual cell volume determination is quite as reliable as is the measurement of the mean diameter by the methods used.

CONCLUSIONS

The determination of the individual cell volume as suggested by Haden and as herein recorded is a more simple means of determining the average cell size than is the measurement of the mean cell diameter.

Determinations of the individual cell volume are very helpful in differentiating pernicious anemia from other anemias in most instances in which difficulty in diagnosis might arise, the outstanding exception being in the rare condition, so-called aleukemic leukemia. During and shortly following acute loss of blood, there is an increase in the average individual cell volume, whereas in anemia resulting from chronic loss of blood, the individual cell volume is generally low.

Figures presented suggest that with adequate liver therapy, the red blood cells of patients with pernicious anemia return to normal volume. An individual cell volume figure greater than normal should indicate inadequate treatment.

Not only is the method for determining the individual cell volume a simple one to use, but the information so obtained is also as reliable as is that obtained by measurement of the mean diameter of the red blood cells by the method used.

Miss Isabel Howard aided with much of the technical work.

IRON METABOLISM IN PERNICIOUS AND IN SECONDARY ANEMIA *

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For a long time it has been known that iron occurs in the blood in some other combination than with hemoglobin, and recent studies [1] have shown that this serum iron varies in quantity during iron starvation in experimental animals. In dogs rendered anemic by long-continued hemorrhage, with a limited intake of iron, the normal level of serum iron is reduced about 33 per cent, and when iron-containing foods or medicinal iron salts are given, the normal level returns, with consequent regeneration of the blood.

The object of this paper is to report clinical studies directed toward the application of these results to the problem of iron metabolism in pernicious anemia and secondary anemia in man.

METHODS

The method for determination of iron devised by Elvehjem and Hart [2] was adopted for this work, since its accuracy for small amounts of iron in biologic materials is unquestioned. One source of error in the determinations of serum iron is that a small amount of hemoglobin will vitiate the readings. This was

TABLE 1.—*Recovery of Iron from 10 Cc. Samples of Serum*

Sample	Initial Fe Content of Serum, Mg.	Added Iron, Mg.	Recovered after Precipitation, Mg.	Error
1	0.1	None	0.1	.....
2	0.1	0.1	0.21	+0.01
3	0.1	0.05	0.15	.....
4	0.1	0.04	0.137	—0.003
5	0.1	0.03	0.129	—0.001

controlled by precipitation of the serum proteins with 10 per cent trichloracetic acid, according to a suggestion of Briggs.[3] Other control studies on the method are reported in a previous paper. The accuracy of the method may be seen from table 1. It is advisable to use not less than 10 cc. samples for analysis, and when this has been done the method has been accurate, in my experience, to 0.02 mg. of iron.

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* From the Department of Medicine of the University of Michigan and the Thomas Henry Simpson Memorial Institute for Medical Research.

1. Riecker, H. H., and Winters, M. E.: Serum Iron Determinations Applied to the Study of Experimental Anemia, Am. J. Physiol. **92**:196 (Feb.) 1930.

2. Elvehjem, C. A., and Hart, E. B.: Iron in Nutrition: II. Quantitative Methods for the Determinations of Iron in Biological Materials, J. Biol. Chem. **64**:43, 1926.

3. Personal communication to the author.

IRON CONTENT OF NORMAL SERUM

In forty persons, I have found the iron content of the serum to be as shown in table 2. From this small group of normal people, the mean value of 1.1 ± 0.022 mg. per hundred cubic centimeters of serum is derived. It is probable that this figure varies somewhat, depending on the method used in determinations of iron, and with this in mind it would be useless to compare my results with those of the earlier investigators.

IRON CONTENT OF SERUM IN PERNICIOUS ANEMIA

With the advent of a method of producing a remission in pernicious anemia by means of liver extract, an opportunity was afforded for determining the iron metabolism in this disease somewhat more completely than had hitherto been possible. A complete study of the iron metabolism in pernicious anemia was made by Queckensted [4] in 1914, in

TABLE 2.—*Values Found for Iron in the Serum of Normal Persons*

Iron per 100 Cc. Serum, Mg.	Persons
0.9	5
1.0	16
1.1	5
1.2	2
1.3	7
1.4	5
	40

which the literature to that date was reviewed. This investigator could find no evidence of destruction of blood by quantitative estimation of the iron in stools and urine in pernicious anemia. However, Kennerknecht [5] in 1911 had found an excess excretion in the stools of several patients with pernicious anemia, amounting to from 15 to 20 mg. per day.

The "control" observations in my cases, preliminary to specific treatment, indicate that the total excretion of iron during relapse is approximately that of normal persons.

It was necessary to determine the iron content of the stools and urine of these patients over a long period of time in order to explain the changes which are found to take place in the level of the serum iron as remission of the disease takes place. Previous studies of the serum iron in pernicious anemia have been reported by Fowell,[6] in which it was

4. Queckensted: Untersuchungen über den Eisenstoffwechsel bei der perniziösen Anämie mit Bemerkungen über den Eisenstoffwechsel überhaupt, Ztschr. f. klin. Med. **79**:49, 1914.

5. Kennerknecht, Klara: Beiträge zur Kenntnis des Eisenstoffwechsels bei perniziösen Anämie und Leukämia, Virchows Arch. f. path. Anat. **205**:89, 1911.

6. Fowell, P. H. C.: Iron in the Blood, Quart. J. Med. **6**:179, 1912.

concluded that the nonhemoglobin iron was much higher than normal, and that this fact might be of value in determining the extent of hemolysis in disease. Erben [7] believed that the nonhemoglobin iron was increased in pernicious anemia, and I have been able to substantiate this in most instances, including that in which phenylhydrazine is used to produce hemolysis.

The serum from nineteen cases of pernicious anemia was analyzed for iron before and after induced remissions. Although there was a wide variation in the initial values, it is believed that in cases for which specific treatment had not been given, the iron levels of the serum were higher than normal. Table 3 gives a summary of the results together with the initial peripheral blood counts. In many of the cases, high

TABLE 3.—*Relation of the Initial Iron Content of the Serum to the Red Blood Cell Count in Pernicious Anemia*

Case	Initial Red Blood Cell Count, Millions	Initial Iron Content of Serum, Mg. per 100 Cc.	Final Values
1	1.4	1.63	1.10
2	1.3	1.3	1.00
3	2.32	1.5	1.4
4	0.73	1.22*	1.2
5	1.99	1.8	1.9
6	2.52	1.2*	1.3
7	1.31	1.0*	1.3
8	1.6	1.1	0.94
9	1.1	1.5	1.0
10	2.12	2.0	1.6
11	1.03	1.08*	0.9
12	1.07	1.17*	1.11
13	1.36	1.1*	0.9
14	1.34	1.33	1.10
15	1.68	1.08*	0.92
16	1.68	1.29	1.00
17	2.08	1.50	1.42
18	3.17	1.42	1.02
19	2.9	1.29	0.90

* Having normal initial values for the serum iron.

initial values of the serum iron became normal when remission was established, while in several the values remained constantly normal throughout the period of observation.

Those of the first group with high initial levels of the serum iron include cases 1 (1.63 mg.), 2 (1.3 mg.), 3 (1.5 mg.), 5 (1.8 mg.), 9 (1.5 mg.), 10 (2 mg.), 14 (1.33 mg.), 16 (1.29 mg.), 17 (1.5 mg.), 18 (1.42 mg.) and 19 (1.29 mg.). In all of these, the level became progressively lower as the blood picture approached normal. However, in some there was not a regular decline in the values of the serum iron, and for this I have no explanation.

Eight of the cases did not show an elevation of serum iron when admitted to the hospital. These are cases 4 (1.22 mg.), 6 (1.2 mg.), 7

7. Erben, F.: Die chemische Zusammensetzung des Blutes bei perniziösen Anämie, Ztschr. f. klin. Med. **40**:267, 1900.

(1 mg.), 8 (1.1 mg.), 10 (1.08 mg.), 11 (1.17 mg.), 12 (1.1 mg.) and 14 (1.08 mg.).

In table 4, the studies of the peripheral blood and the corresponding determinations of serum iron have been compared. The abnormally high initial values of the serum iron bring to mind the corresponding increased bilirubin in the blood serum and suggest the possibility that these substances are derived from the same source, that of hemolysis of immature erythrocytes in the bone-marrow.

TABLE 4.—*Iron Content of the Serum in Pernicious Anemia*

Patient	Date	Red Blood Cells	Hemoglobin (Sahli), %	Reticulocytes, %	Fe in Serum, Mg. per 100 Cc.	Medication
1	June 22	1,400,000	31	....	1.63	
	June 26	.....	..	....	1.60	Hog stomach
	June 28	.....	..	....	1.8	
	July 1	.....	..	....	1.9	
	July 8	2,490,000	45	....	1.6	
	July 10	.....	..	....	1.4	
	July 12	.....	..	....	1.3	
	July 15	2,690,000	58	....	1.2	
	July 17	.....	..	....	1.0	
	July 22	3,390,000	62	....	1.12	
	July 29	.....	..	....	1.2	
	Aug. 2	3,400,000	69	....	1.2	
	Aug. 5	.....	..	....	1.15	
	Aug. 9	.....	..	....	1.12	
	Aug. 12	.....	..	....	1.15	
	Aug. 15	4,660,000	77	....	1.10	
2	Feb. 2	1,300,000	28	1.0	....	
	Feb. 9	.....	..	....	1.3	
	Feb. 11	.....	..	....	1.3	Lilly liver extract
	Feb. 13	.....	..	....	1.25	
	Feb. 14	.....	..	....	1.3	
	Feb. 16	.....	..	21.3	1.0	
	Feb. 18	1,630,000	27	3.5	....	
	March 11	3,140,000	52	....	....	
3	Jan. 7	2,320,000	54	0.5	1.5	
	Jan. 12	2,050,000	47	1.9	1.25	Lilly liver extract
	Jan. 14	.....	..	3.7	1.3	
	Jan. 18	2,970,000	67	7.1	1.4	
	Jan. 20	.....	..	6.6	....	
	Jan. 24	3,200,000	70	3.0	1.4	
4	July 2	730,000	19	1.5	....	
	July 6	.....	..	....	1.22	Lilly liver extract
	July 10	1,060,000	26	14.9	1.20	
	July 12	.....	..	....	1.33	
	July 15	1,370,000	28	11.8	1.2	
5	Aug. 5	1,990,000	23	1.5	....	Hog stomach
	Aug. 9	.....	..	....	1.8	
	Aug. 12	.....	..	....	1.9	
	Aug. 20	2,250,000	41	....	....	
6	July 10	2,520,000	52	0.4	....	
	July 12	.....	..	....	1.2	
	July 19	1,890,000	55	3.8	1.1	
	July 22	.....	..	....	1.3	
	July 23	1,780,000	48	5.9	....	
7	Feb. 15	1,310,000	25	....	1.0	
	Feb. 18	1,300,000	27	....	1.0	Lilly liver extract
	Feb. 20	.....	..	....	0.9	
	Feb. 23	.....	..	....	1.1	
	Feb. 25	1,620,000	40	....	1.2	
	Feb. 27	.....	..	....	1.3	
	March 2	2,350,000	51	....	1.3	
8	July 15	1,600,000	32	....	1.1	Hog stomach
	July 17	.....	..	....	1.1	
	July 19	2,160,000	43	....	1.3	
	July 22	.....	..	....	1.23	
	July 29	3,240,000	62	....	0.94	

TABLE 4.—*Iron Content of the Serum in Pernicious Anemia—Continued*

Patient	Date	Red Blood Cells	Hemoglobin (Sahli), %	Reticulocytes, %	Fe in Serum, Mg. per 100 Cc.	Medication
9	Feb. 12	1,100,000	21	2.0	1.5	Lilly liver extract
	Feb. 14	1,250,000	25	2.3	0.9	
	Feb. 18	.....	..	9.5	1.3	
	Feb. 21	2,120,000	34	25.9	1.1	
	Feb. 25	.....	..	....	0.9	
	Feb. 27	2,340,000	38	....	0.875	
	March 1	.....	..	....	0.9	
	March 9	3,400,000	65	....	1.0	
10	June 12	2,120,000	50	0.8	....	Lilly liver extract
	June 19	.....	..	....	2.0	
	June 24	2,340,000	46	6.9	1.43	
	June 26	3,080,000	55	5.7	1.6	
11	Nov. 19	1,030,000	27	2.3	1.08	
	Nov. 22	.....	..	....	1.09	
	Nov. 25	1,200,000	24	2.2	1.08	
	Nov. 29	.....	..	....	0.98	Hog stomach
	Dec. 3	1,080,000	22	7.4	0.90	
12	Sept. 10	1,070,000	20	2.0	1.17	
	Sept. 12	.....	..	1.1	1.17	
	Sept. 14	.....	..	1.6	1.19	
	Sept. 18	890,000	20	0.7	1.22	
	Sept. 20	.....	..	0.8	1.23	
	Sept. 23	.....	..	3.1	1.22	
	Sept. 26	920,000	20	9.1	1.5	
	Sept. 30	700,000	18	1.6	1.3	
	Oct. 2	.....	..	1.0	1.04	
	Oct. 4	.....	..	1.3	1.02	
	Oct. 7	68,000	17	0.9	1.02	
	Oct. 11	.....	..	....	1.02	Hog stomach
	Oct. 14	98,000	20	28.1	1.02	
	Oct. 19	.....	..	0.9	1.11	
	Oct. 22	1,350,000	34	1.0	1.13	
	Oct. 25	1,510,000	33	2.1	1.11	
13	Oct. 11	1,360,000	30	1.6	1.1	Hog stomach
	Oct. 19	1,280,000	30	0.5	1.12	
	Oct. 22	.....	..	2.0	1.0	
	Oct. 25	.....	..	....	0.89	
	Oct. 26	1,520,000	36	17.0	....	
	Oct. 29	1,780,000	42	12.8	1.13	
	Nov. 4	.....	..	1.8	1.13	
	Nov. 5	2,100,000	50	2.2	....	
14	Nov. 15	700,000	17	1.4	....	Lilly's liver extract
	Nov. 22	1,340,000	20	40.9	1.33	
	Nov. 25	.....	..	21.4	1.33	
	Nov. 29	2,400,000	39	9.3	1.10	
	Dec. 2	3,160,000	44	8.2	....	
15	Nov. 20	1,680,000	42	3.5	1.08	
	Nov. 22	.....	..	3.7	1.07	
	Nov. 25	1,160,000	34	2.0	1.08	Hog stomach
	Nov. 29	.....	..	2.7	0.9	
	Dec. 2	1,430,000	39	5.7	0.92	
16	Nov. 20	1,680,000	32	2.3	1.29	Parke Davis & Co.
	Nov. 22	.....	..	1.3	1.29	liver extract
	Nov. 25	2,010,000	44	19.3	1.28	
	Nov. 29	.....	..	....	1.00	
	Dec. 2	2,660,000	49	4.9	....	
17	Sept. 4	2,080,000	42	15.2	1.50	Lilly's liver extract
	Sept. 6	.....	..	18.5	1.50	
	Sept. 9	.....	..	....	1.50	
	Sept. 14	2,600,000	61	3.1	1.46	
	Sept. 16	2,880,000	61	0.7	1.42	
18	Sept. 6	.....	..	....	1.42	Lilly's liver extract
	Sept. 9	3,170,000	65	5.1	1.3	
	Sept. 4	.....	..	0.9	1.20	
	Sept. 16	3,640,000	65	1.4	1.03	
	Sept. 20	4,130,000	79	....	1.02	
19	Sept. 30	2,900,000	65	6.7	1.29	
	Oct. 2	.....	..	....	1.26	
	Oct. 4	2,830,000	64	1.9	1.20	
	Oct. 7	2,740,000	57	0.1	1.20	Lilly's liver extract
	Oct. 14	3,080,000	68	7.7	0.90	

METHOD FOR DETERMINATION OF IRON METABOLISM IN PERNICIOUS ANEMIA

For determination of the iron metabolism in pernicious anemia, six patients were selected who had been given no specific treatment. They were placed in bed under strict surveillance as to food and water intake and the collection of specimens. A diet was designed to contain 7.5 mg. of iron based on Shermans' analyses, and the foods prepared with distiller water.[8]

After a control period of between four and eleven days on the diet, the usual remission with liver extract was induced.

Stools were collected in white porcelain pails, pooled into five day lots (except when otherwise indicated) and dried over a steam bath. The total pooled specimen was then removed by a hard-wood spatula, the container being scraped with a porcelain spatula, and the specimen weighed. It was then ground to dust in a large porcelain mortar over which a rubber membrane had been attached. From this a sample was taken for analysis.

Collections of urine were made in the usual manner, pooled, measured, and samples analyzed corresponding to the collections of stools.

During the progress of the remission, it was necessary to increase the caloric value of the diet; this was done in a definite manner, and the increased content of iron was noted.

In the case of the two female patients, the collections of urine were not complete, but even here the results differed only in degree from those in the remainder of the series.

On several occasions, duplicate diets were prepared; one was fed to the patient and the other was dried, ground and analyzed for iron as a control measure. The results for calculated and chemically determined iron were close.

A patient with mild arthritis deformans was placed on the standard regimen, and the intake and the excretion of iron were found to balance during a ten day period. In none of the patients was there occult blood in the stools. Duplicate samples from the specimens of stools and urine were analyzed, and the results for iron used in derivation of the average figure, and in all cases medication of any nature was noted and analyzed and the result for iron added to the iron intake.

IRON METABOLISM IN PERNICIOUS ANEMIA

Case 1 was followed for a period of seventy-one days, during which the hemoglobin level (Sahli) rose from 28 per cent to 78 per cent, and the erythrocyte count from 1,020,000 to 4,570,000. During this period, the patient excreted 1,274.74 mg. of iron. His total intake of iron as food and in the liver extract was 634.28 mg., giving an excess excretion of 640.36 mg. The excretion was not constant and appeared greatest at the beginning of the second half of the period, that is, one month after treatment had begun.

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8. Peterson, W. H., and Elvehjem, C. A.: Iron Content of Plant and Animal Foods, J. Biol. Chem. **78**:215 (June) 1928. Sherman, Henry: Chemistry of Food and Nutrition, New York, The Macmillan Company, 1927.

In case 2, the determinations were carried out over a period of thirty-four days; the hemoglobin level increased from 25 per cent to 88 per cent. The disease was of shorter duration than that of the previous patient and the patient was younger. He also had an exophthalmic goiter. However, the excess excretion was 297.3 mg., while the

TABLE 5.—*Iron Excretion During Active Remission in Case 1*

Dates, 1929	Excretion of Iron in Stool, Mg.	Excretion of Iron in Urine, Mg.	Total Excretion for Period, Mg.	Average Daily Excretion, Mg.	Food Iron Intake Daily, Mg.	Daily Excess Excretion, Mg.
Aug. 28-29	8.31	6.77	15.10	7.55	7.5	0.50
Aug. 30-31	8.06	8.05	16.11	8.05	7.5	1.55
Treatment with Liver Extract Began						
Sept. 1-5	26.89	21.26	45.15	9.63	7.5	2.13
Sept. 6-10	46.00	36.01	82.01	16.40	7.5	8.90
Sept. 11-15	63.27	37.88	101.15	20.23	7.5	12.70
Sept. 16-20	82.22	29.46	111.68	22.33	9.5	12.83
Sept. 21-25	36.56	27.54	64.10	12.82	10.0	2.82
Sept. 26-30	42.10	27.98	70.08	14.00	11.0	3.00
Oct. 1-5	37.98	42.26	80.24	16.05	12.5	3.55
Oct. 6-10	78.54	43.36	121.90	22.40	12.5	9.90
Oct. 11-15	71.96	30.83	102.79	20.56	12.5	8.06
Oct. 16-20	47.32	31.41	78.73	15.74	12.5	3.20
Oct. 21-25	66.70	36.88	103.58	20.71	10.0	10.71
Oct. 26-30	32.90	98.96	131.86	26.40	10.0	16.40
Oct. 31-Nov. 4	62.66	26.00	88.66	17.73	10.0	7.73
Nov. 5-7	39.72	18.84	58.56	19.52	10.0	9.52
Totals †	751.19	523.49	1,274.74		575.0	699.77

† Hemoglobin level rose from 28 per cent to 78 per cent and the red blood cell count from 1,020,000 to 4,570,000.

Iron in Lilly extract, 59.28 mg., subtracted from total excretion leaves excess excretion of 640.42 mg.

TABLE 6.—*Iron Excretion During Active Remission in Case 2*

Dates, 1929	Excretion of Iron in Stool, Mg.	Excretion of Iron in Urine, Mg.	Total Excretion for Period, Mg.	Average Daily Excretion, Mg.	Food Iron Intake Daily, Mg.	Daily Excess Excretion, Mg.
Oct. 26-30	59.2	32.7	31.9	18.40	7.5	10.9
Oct. 31-Nov. 4	44.9	20.9	65.8	13.60	7.5	5.6
Nov. 5-9	27.4	43.4	70.8	14.16	7.5	6.6
Nov. 10-14	40.1	43.3	83.4	16.70	8.0	8.7
Nov. 15-19	48.0	33.0	81.0	16.02	10.0	6.0
Nov. 20-24	84.9	31.6	116.5	25.30	12.0	13.3
Nov. 25-28	77.7	34.6	112.3	28.08	12.5	16.5
Totals for 34 days †	382.2	239.5	621.7		312.5	309.2

† Hemoglobin level rose from 25 per cent to 88 per cent and the red blood cell count from 940,000 to 5,600,000.

Iron in Lilly extract, 11.85 mg., subtracted from total excretion leaves excess excretion of 297.35 mg.

total iron intake was 324.3 mg. He was still excreting large quantities of iron when referred for operation for exophthalmic goiter.

Case 3 was observed for a period of eighteen days, four of which were in the control period. The hemoglobin level rose from 40 per cent to 50 per cent. The patient's excess excretion of iron amounted to 107.45 mg. The marine liver extract used in this case contained 275

mg. of iron. On account of the short period of observation there was a low excess excretion of iron.

In case 4, observed for twenty-three days, an excess excretion of 162.38 mg. of iron was obtained, although the patient had not been under observation a sufficiently long time to obtain a true metabolic picture.

Case 5 was observed during a fifty-one day period. The collections of urine were incomplete throughout, but an excess excretion of 126.62 mg. of iron was found. The liver extract contained 343.95 mg. of iron,

TABLE 7.—*Iron Excretion During Active Remission in Case 3*

Dates, 1929	Excretion of Iron in Stool for Period, Mg.	Excretion of Iron in Urine for Period, Mg.	Total Excretion for Period, Mg.	Average Daily Excretion, Mg.	Average Food and Medicine Iron Intake Daily, Mg.	Average Daily Excess Excretion, Mg.
Feb. 22-23-24	31.6	8.70	40.30	13.40	8.71	4.69
Feb. 25	8.4	8.80	17.20	17.20	9.43	17.77
	Treatment with Liver Extract Began					
Feb. 26-March 2	107.9	14.30	122.20	24.40	23.76	0.64
March 3-7	141.0	17.80	158.80	31.76	26.01	5.75
March 8-13	159.6	33.49	193.09	38.62	27.93	10.69
Totals †	448.5	83.09	531.59		424.06	107.43

† Hemoglobin level rose from 40 per cent to 50 per cent and the red blood cell count from 1,410,000 to 2,180,000.

Marine liver extract used, containing 275.4 mg. iron.

TABLE 8.—*Excretion of Iron in Case 4*

Dates, 1929	Excretion of Iron in Stool for Period, Mg.	Excretion of Iron in Urine for Period, Mg.	Total Excretion for Period, Mg.	Average Daily Excretion, Mg.	Average Daily Food Iron Intake, Mg.	Average Daily Excess Excretion, Mg.
March 19-21	68.50	9.50	78.00	26.00	9.67	16.33
March 22-24	29.40	15.00	44.40	13.93	9.94	3.99
March 25-27	39.00	8.10	47.10	15.70	10.22	5.48
March 28-30	24.60	12.10	36.70	12.23	10.62	1.61
March 31-April 2	33.40	12.84	36.24	12.08	9.56	2.52
April 3-7	81.76	25.70	107.46	21.49	10.80	10.69
April 8-12	54.12	21.26	75.38	15.07	12.18	2.89
Totals †	320.78	104.50	425.28		246.94	178.34

† Hemoglobin rose from 51 per cent to 63 per cent and the red blood cell count from 2,020,000 to 2,540,000.

Iron in liver extract, 15.96 mg.; subtracted leaves excess excretion, 162.38 mg.

and under a more rigidly reduced intake of iron a much higher excretion would have been obtained.

Case 6 was observed forty-six days, during which the hemoglobin level rose from 24 to 67 per cent. The excess excretion of iron was 196.76 mg. The elimination of relatively large amounts of iron during remission agrees with the recent observation of pathologists that hemosiderosis has been conspicuously absent in the tissues of patients dying during a remission induced by liver extract.

IRON METABOLISM IN SECONDARY ANEMIA

Studies of the serum iron were made on twenty cases of various types of secondary anemia and two cases of polycythemia vera.

An attempt was made to answer the following questions: 1. Is there evidence of iron starvation in certain types of secondary anemia? 2. Does

TABLE 9.—*Iron Excretion During Active Remission in Case 5*

Dates, 1929	Excretion of Iron in Stool for Period, Mg.	Excretion of Iron in Urine for Period, Mg.*	Total Excretion for Period, Mg.	Average Daily Excretion, Mg.	Average Daily Food Iron Intake, Mg.	Average Daily Excess Excretion, Mg.
Jan. 31-Feb. 4	33.95	15.90	49.85	9.97	6.76	3.21
Feb. 5-6	24.16	8.60	32.76	16.38	6.45	9.93
	Treatment with Liver Extract Began					
Feb. 7-10	56.90	14.38	71.28	17.82	5.95	11.87
Feb. 11-15	42.30	11.84	54.14	10.83	6.82	4.01
Feb. 16-20	49.95	18.42	68.37	13.67	7.03	6.64
Feb. 21-25	106.70	24.70	131.40	26.26	6.90	19.36
Feb. 26-March 2	67.90	13.70	81.60	16.32	6.38	9.94
March 3-7	68.30	13.95	82.25	16.45	6.17	10.28
March 8-12	58.20	15.59	73.79	14.76	8.19	6.57
March 13-17	63.60	16.25	79.85	15.97	8.43	7.54
March 18-22	84.40	17.45	101.85	20.37	10.19	9.88
March 23-28	56.00	28.10	84.10	14.01	11.25	2.76
Totals †	712.36	198.88	911.24		440.67	470.57

* Urine collection incomplete throughout.

† Hemoglobin level rose from 40 per cent to 79 per cent and the red blood cell count rose from 1,800,000 to 4,470,000.

Iron in liver extract, 343.95 mg., subtracted leaves 126.62 mg. excess excretion.

TABLE 10.—*Excretion of Iron in Case 6*

Dates, 1929	Excretion of Iron in Stool for Period, Mg.	Excretion of Iron in Urine for Period, Mg.*	Total Excretion of Iron for Period, Mg.	Average Daily Iron Excretion, Mg.	Average Daily Food Iron Intake, Mg.	Average Daily Iron Excess, Mg.
Feb. 15-19	89.54	23.03	112.57	22.51	5.78	16.73
Feb. 20-24	57.90	21.25	79.15	15.83	5.76	10.07
	Treatment with Liver Extract Began					
Feb. 25-March 1	20.10	25.52	45.62	9.12	5.87	3.25
March 2-6	41.40	18.76	60.16	12.03	6.30	5.73
March 7-11	54.40	15.55	69.95	13.99	8.54	5.45
March 12-16	33.60	9.95	43.55	8.71	10.05	—1.34
March 17-21	104.50	16.38	120.88	24.18	9.80	14.38
March 22-26	87.00	21.72	108.72	21.74	9.59	12.15
March 27-April 1	57.90	23.50	81.40	13.56	9.94	3.62
Totals †	546.34	175.66	766.00			353.87

* Collection of urine incomplete throughout.

† Hemoglobin rose from 24 per cent to 67 per cent and the red blood cell count from 1,000,000 to 3,530,000.

Iron in liver extract, 157.08 mg.; subtracted leaves excess excretion, 196.79 mg.

iron medication influence the level of the serum iron? 3. Is there a relationship between the level of the serum iron and that of hemoglobin in these conditions?

Iron starvation most likely would be found in patients presenting a history of long-continued bleeding from the gastro-intestinal tract. How-

ever, a large number fail to show it because iron medication or a diet rich in iron had been used in their treatment. I have studied two cases of peptic ulcer, nos. 3 and 10, with initial values of serum iron below the average normal and almost as low as in experimental animals under

TABLE 11.—*Iron Content of the Serum in Secondary Anemias*

Patient		Date	Red Blood Cells	Hemoglobin (Sahli), %	Reticulocytes, %	Fe in Serum Mg. per 100 Cc.	Medication
1	Myxedema	Sept. 23	3,300,000	52	...	1.94	Thyroid extract
		Sept. 25	3,210,000	52	...	1.7	
2	Secondary anemia; gallstones	July 2	3,310,000	43	...	1.0	10% solution ferric ammonium citrate three times a day
		July 8	3,670,000	46	...	1.26	
		July 12	3,310,000	54	...	1.3	
		July 23	4,500,000	48	...	1.45	
		Aug. 6	4,890,000	61	2.8	....	
3	Duodenal ulcer	May 9	3,210,000	65	...	....	"High iron" diet
		May 11	.....	..	...	0.9	
		May 14	4,100,000	71	...	0.85	
		May 17	4,380,000	70	...	1.0	
4	Rectal bleeding	Oct. 19	.....	..	...	1.09	Iron medication
		Oct. 25	.....	..	...	1.33	
		Nov. 1	.....	..	...	1.29	
5	Menorrhagia	June 3	4,270,000	32	...	2.4	4 Gm. ferric ammonium citrate daily
		June 6	4,230,000	33	...	2.6	
		June 8	.....	..	...	1.4	
		June 14	4,300,000	33	...	1.6	
6	Cancer of cecum	Feb. 2	2,950,000	20	...	1.0	None
		Feb. 4	2,590,000	15	...	0.94	
		Feb. 6	.....	..	...	1.0	
		Feb. 8	2,310,000	15	...	1.3	
7	.....	March 15	2,630,000	26	...	1.1	No iron
8	.....	June 24	1,660,000	20	...	1.18	50% solution of ferric ammonium citrate, 4 cc. three times daily
		July 8	2,700,000	31	...	1.6	
9	Cancer of stomach	May 29	2,710,000	30	...	1.33	Iron medication as above
		June 9	.....	20	...	1.4	
10	Bleeding septic ulcer	April 25	2,780,000	45	2.0	0.8	Began with 50% solution of ferric ammonium citrate, 8 cc. three times daily
		April 26	.....	..	...	....	
		April 27	2,780,000	41	1.5	0.8	
		April 29	2,910,000	37	1.9	0.8	
		May 3	3,220,000	42	2.1	0.9	
		May 6	3,870,000	43	3.2	1.0	
		May 13	4,160,000	51	3.4	1.2	
11	Duodenal ulcer	May 10	3,350,000	50	...	....	Ferric ammonium citrate on May, 20, 1929
		May 11	.....	..	...	1.5	
		May 24	.....	..	...	1.7	
		May 31	.....	..	...	1.7	
		June 3	2,000,000	40	...	....	
12	Aplastic anemia	Jan. 19	660,000	16	...	1.3	Blaud's pills; transfusions
		Jan. 22	640,000	15	...	1.2	
		Jan. 28	730,000	15	...	1.2	
		Feb. 1	.....	..	...	1.2	
		Feb. 3	.....	..	...	1.1	
		Feb. 6	850,000	31	...	1.1	
13	Ulcer of stomach	Feb. 16	1,910,000	22	...	1.7	Ferrous iodide 4 cc. three times daily; discontinued; transfusions Feb. 17 and 21
		March 4	2,700,000	50	...	1.5	
		March 8	.....	..	...	1.3	
		March 15	.....	..	...	1.1	
14	Cancer of stomach	Feb. 4	1,940,000	15	...	1.9	Transfusion
		Feb. 6	2,650,000	24	...	1.8	
		Feb. 9	.....	..	...	1.0	
		Feb. 11	.....	..	...	1.0	
		Feb. 15	2,980,000	26	...	1.5	
15	.....	Jan. 10	.....	40	...	1.55	Iron; blaud's pills
		Jan. 14	3,650,000	41	...	1.24	
		Jan. 22	3,270,000	40	...	1.3	
		Feb. 7	3,730,000	42	...	1.4	
		Feb. 20	4,150,000	52	...	1.4	

similar conditions. The first case gave an initial value of 0.9 mg. and the second 0.8 mg., and under iron medication these figures became normal or above normal. It might be supposed that the serum iron increased as the patient improved, but in other cases an initial high value of iron was found, and besides in the experimental anemia no such relationship was apparent.

Of two patients with polycythemia, one had a high level of the serum iron (1.80, 1.75) while under treatment with phenylhydrazine, and the other a level much below the average normal (0.86 mg.). The latter was treated by repeated venesection while on a diet containing a minimum amount of iron. These values are interesting but capable of anticipation in view of the contrasting forms of treatment utilized.

In a case of menorrhagia (no. 5; hemoglobin 32 per cent, red blood cells 4,270,000) and one of duodenal ulcer (no. 11; hemoglobin 50 per cent, red blood cells 3,350,000) for which iron had been given as medication, the levels of the serum iron were much higher than normal, thus indicating that the iron content of the serum can be materially raised by such a procedure. In case 4, the level of the serum iron was raised from a normal of 1.09 to 1.33 mg. by iron as medication.

Of four children with so-called nutritional anemia, two were found to have low and two normal levels of the serum iron. They all improved without the use of medicinal iron.

A summary of the data for the cases of secondary anemia is given in table 11.

COMMENT

Determination of the iron content of the blood serum may have practical clinical value in all forms of anemia, and various types of medication may be studied with reference to absorption by this means. It is believed that the color index should be of value in determining the necessity for medicinal iron, and in my experience patients with low color indexes respond best, and these are most often found to have relatively low levels of the serum iron. However, such conditions as carcinomatosis, with a low color index, show a high level of the serum iron, and in these medicinal iron can have no value.

It is known, then, that clinical, as well as experimental, iron starvation is reflected in the level of the serum iron, and that feeding iron in any form increases this level. This conclusion is based only on clinical evidence, for when iron is administered in the experimental anemia of hemorrhage, regeneration of hemoglobin results immediately. I can find no direct relationship between the level of the serum iron and that of hemoglobin, since this is governed by other factors. However, it might be said that cases of secondary anemia due to iron starvation are comparatively rare, and that the indication for medicinal iron likewise

is seldom present. Unpublished data in my possession indicate that the form or compound of iron used has no appreciable significance and that animal experiments purporting to show this cannot be successfully applied clinically.

It is not known in what form the iron occurs which is not combined as hemoglobin, and Fowell [6] discussed this problem in his excellent study. He believed that minute quantities of inorganic iron are highly toxic in the blood stream and assumed that the compound must be in either colloidal or organic form. I have analyzed spinal fluid and found just an appreciable amount of iron (0.4 mg. per hundred cubic centimeters), hence it might be presumed that the serum iron is partially nondiffusible. That it is not in combination with protein is shown by the method of precipitating the protein of the serum before making the analyses. It is possible that several compounds are present. The problem has interesting possibilities.

CONCLUSIONS

1. There is present in blood serum a partly diffusible iron compound the estimation of which may be used to determine the presence and degree of iron starvation in disease and likewise the effect of iron medication.

2. The amount of serum iron is increased in hemolytic diseases of the blood and decreased in the presence of long continued hemorrhage or a dietary deficiency of iron.

3. In pernicious anemia, the level of the serum iron usually is higher than normal but becomes normal during remissions in the disease.

4. A remission in pernicious anemia is accompanied by an increase in excretion over the intake of iron, as determined by analyses of stool and urine.

5. In secondary anemia, one rarely finds as marked iron starvation as in experimental hemorrhage in animals on a diet low in iron, and from this standpoint alone, the indications for medicinal iron are limited.

THE SIGNIFICANCE OF AN ELECTROCARDIOGRAM WITH A LARGE Q IN LEAD 3*

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The presence of a large Q wave in the third lead is frequently observed in electrocardiographic records from patients with the anginal syndrome. In the majority of these records the QRS group shows left axis deviation, though in a few the electrical axis has a normal direction. The frequency of the large Q-3, both in cardiac patients in general and in those with the anginal syndrome in particular, will appear from the following: In a series of 200 cases of heart disease of various sorts recorded alphabetically in a file of office patients, 30 showed a diagnosis of the anginal syndrome, including both the angina of effort and the angina appearing at rest.[1] Of these 30 patients with the anginal syndrome 8, or 27 per cent, were found to have a large Q wave in the third lead. In the other 170 cases 6 such records were found; so that the peculiarity in question occurred in patients with heart disease without the anginal syndrome with a frequency of only 3.5 per cent.

In any discussion of the height of one of the QRS deflections one must take into account the maximum voltage of the QRS group as shown by the amplitude of the largest wave of this group in whichever lead this may occur. For example, the current producing a Q wave of 3 mm. in lead 3 is a less important part of a QRS group the largest excursion of which is an R-2 of 18 mm. than it is of one the largest deflection of which is an R-2 of 5 mm. In the first case the Q bears only an insignificant relation to the largest current of QRS, while in the second case the current producing Q is more than half as large as the maximum produced. It became necessary, then, to decide what should be considered a large Q wave. After considerable inspection and examination of various records, it seemed that if 25 per cent of the maximum excursion of QRS, in whichever lead this appeared, were considered as the dividing line between large and small Q waves, there would be little danger of applying the term abnormal to a wave that was really normal. In examining the records of fifty-two normal persons,

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* From the New York Hospital and the Department of Medicine of the Cornell University Medical School.

1. Vaquez, H., and Laidlaw, George F.: Diseases of the Heart, Philadelphia, W. B. Saunders Company, 1924. Criteria Committee of New York Tuberculosis and Health Association: Criteria for the Classification and Diagnosis of Heart Disease, New York Tuberculosis and Health Association, 1929.

Lewis and Gilder[2] found that the largest Q wave in lead 3 measured 2.5 mm. The amplitude of Q-3 in this series was always less than 25 per cent of the amplitude of the largest R of the record.

In addition to a Q wave in the third lead that was more than 25 per cent of the largest excursion of QRS, the records under consideration (fig. 1) show either left axis deviation of QRS with R-1 greater than R-2 or, more rarely, a normal direction of the axis of QRS with R-2 larger than either R-1 or R-3. Records showing R-3 larger than R-2 were not included in this study, for Q-3 is a normal feature of records showing right axis deviation. In certain records there is a small Q in

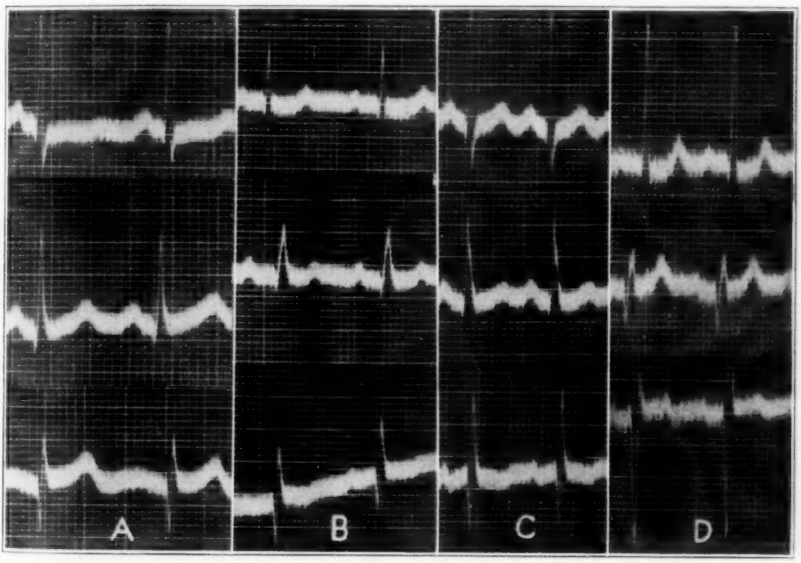

Fig. 1.—Four of the records showing a large Q wave in lead 3.

lead 1, and possibly in lead 2 as well; often Q-2 is larger than Q-1. There is often a fair-sized S wave in lead 1, and when S is absent from lead 1 it does not appear in lead 2. The least frequent of these waves is S-2 (8 times). The others appear with about equal frequency: Q-1, 21 times; S-1, 29 times, and Q-2, 27 times. The most frequent combinations are Q-1, S-1 and Q-2 (11 times), S-1 and S-2 (8 times) and Q-1 and Q-2 (5 times); no Q or S appears in either lead in 4 instances.

2. Lewis, T., and Gilder, M. D. D.: The Human Electrocardiogram: A Preliminary Investigation of Young Adults to Form a Basis for a Pathological Study, Phil. Tr. Roy. Soc. London **202**:351 (March) 1912.

The T wave in these records is often normal (63 per cent of the forty-three studied); either upward in all leads (28 per cent) or upward in leads 1 and 2 and downward in lead 3 (35 per cent). It is often found upward in lead 1 and downward in leads 2 and 3 (25 per cent), and occasionally downward in leads 1 and 2 and upward in lead 3 (9 per cent). One patient (2.5 per cent) had a downward T-1, with an upward T-2 and T-3; none had a downward T in all leads. It is an interesting fact that of the sixteen patients (see accompanying table) with a large Q-3 who did not have the anginal syndrome, only one had an abnormal T wave (this was downward in leads 1 and 2, and upward

Frequency of Large Q-3 in Patients with Cardiac Disease

	Large Q-3	Small Q-3
Anginal syndrome	27 (63%)	2 (7%)
Syphilitic aortic insufficiency	1	0
Myocardial fibrosis		
With congestion	4	0
With arhythmia	1	0
Rheumatic heart disease		
Active, with mitral insufficiency	1	0
Aortic insufficiency, with or without mitral disease	2	3
Mitral insufficiency	0	2
Mitral stenosis, with or without mitral insufficiency	1	3
Arteriosclerosis with hypertension	1	2
Hypertension with arhythmia	1	2
Hyperthyroid (enlarged heart), enlargement on percussion and by roentgenograms; patient 54 years of age	1	0
Pregnancy, a record after delivery did not show a large Q-3 (normal heart)	1	0
Effort syndrome, including the group of cases showing neurocirculatory asthenia (normal heart)	2	7
Normal heart	0	5
Congenital heart defect	0	1
Arteritis obliterans	0	1
	43	28

in lead 3). The greater frequency of inversion of T-2 and T-3 (25 per cent) as compared with T-1, with or without T-2 (11.5 per cent), is noteworthy.

Through Dr. A. E. Cohn it was possible to review the electrocardiograms of 160 normal soldiers [3] taken at the Hospital of the Rockefeller Institute. Four records were found with a large Q-3 of a value of 25 per cent or more of the largest wave of the QRS group. Two of these showed right axis deviation of QRS and so were not the type of record here under consideration. The other two were of the type shown in figure 1; one of them had slight left axis deviation of QRS and an inverted T-3, while the other showed a normal direction of the electrical axis with T upward in all leads. Another series of records of sixty-five normal hearts, taken at the clinic of the Cornell Medical School, was

3. Cohn, A. E.: An Investigation of the Size of the Heart in Soldiers by the Teleroentgen Method, Arch. Int. Med. **25**:499 (May) 1920.

made available by Dr. A. M. Master. No records with a large Q-3 were found in this group, nor was there any in the fifty-two records of Lewis' series [2] which he loaned to me for examination. Thus, only two records of this peculiar type were found in the 277 records from normal hearts.

In the course of selecting these records, many were found of the type seen in figure 2, with a downward deflection in lead 3 followed by an upward deflection and then another downward deflection, so that in this lead the QRS group had somewhat the shape of the letter W. Other records were observed in which there was a small upward deflection

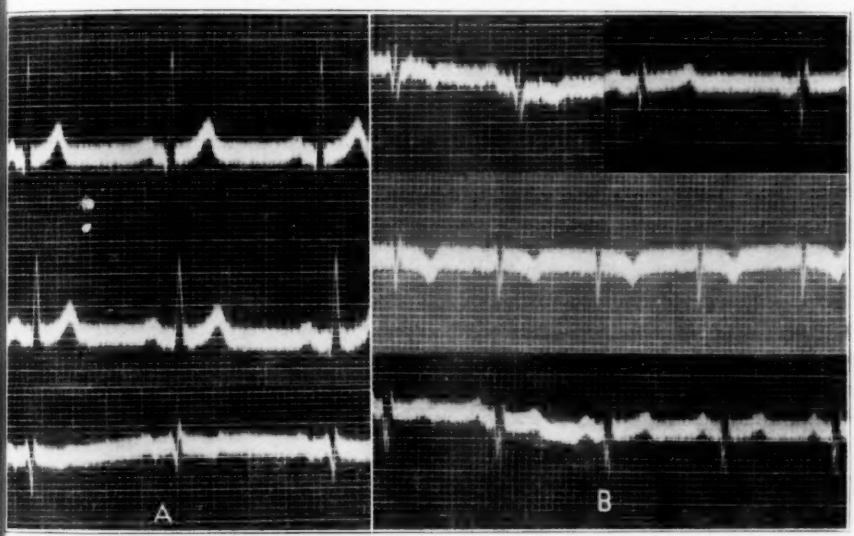

Fig. 2.—*A*, record showing the M type of complex in lead 3; *B*, lead 3 of four records, showing M or W type of complexes.

followed by a larger downward deflection and then another upward deflection, so that the QRS had somewhat the shape of a deformed letter M. In other records the QRS in lead 3 was even more definitely vibratory. All such records were excluded, the only ones considered being those that conformed strictly to the type seen in figure 1, having in lead 3 an initial downward deflection (Q) followed by a definite upward deflection (R), without an S wave.

For comparative study a group of twenty-eight records was selected from the same file, having a small Q wave in lead 3 according to the arbitrary standard used, i.e., less than 25 per cent of the maximum excursion of QRS in whichever lead this appeared, but which conformed

to the other features described for the records with a large Q-3. These records (fig. 3) showed either left axis deviation or QRS or a normal direction of the axis; they often had an S in lead 1, but never an S in lead 3. Q was present in lead 1, lead 2 or both. Such records were perhaps two and one-half or three times as frequent as those showing a large Q-3.

A comparison of the clinical diagnoses in forty-three cases showing a large Q-3 with the diagnoses in twenty-eight cases with a small Q-3 appears in the accompanying table. It will be seen that 63 per cent of the patients showing a large Q-3 were found to be suffering from the

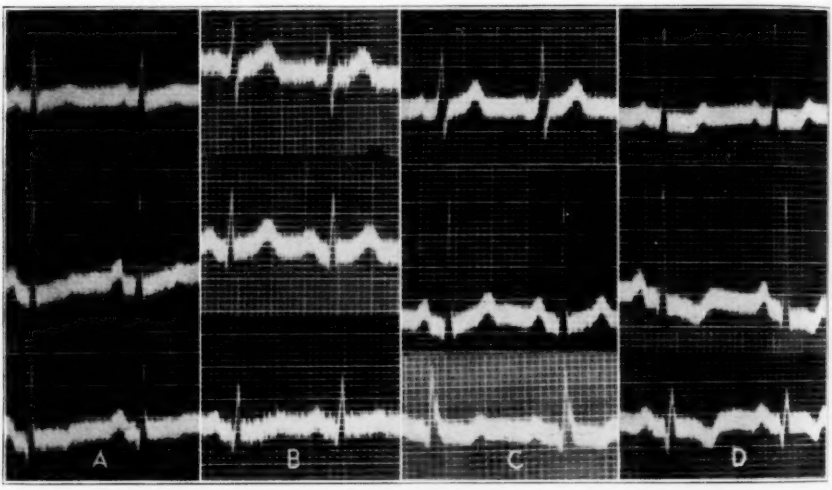

Fig. 3.—Four records showing a small Q wave in lead 3.

anginal syndrome, whereas only 7 per cent of those with a small Q-3 had this complaint. The pathologic changes underlying the anginal syndrome are admittedly various, but it is generally agreed that occlusion, or at least marked narrowing, of a coronary branch is the most frequent cause of the angina of rest, and that coronary narrowing without occlusion is probably the most frequent cause of the angina of effort. A review of the other diagnoses in the table reveals syphilitic aortitis with aortic insufficiency, myocardial fibrosis and rheumatic aortic insufficiency, all conditions that might well be associated with coronary narrowing. If the eight cases with these diagnoses are added to the twenty-seven with an anginal syndrome, one has a total of thirty-five, or 81 per cent of the group with a large Q-3. In this 81 per cent the underlying pathologic process is probably the narrowing of a coronary

branch or branches. If one adds together the same types of cases in the group with a small Q-3 one has five, or 17 per cent of the total.

If one selects from the group with a large Q-3 those which showed a Q wave with a value of 50 per cent or more of the maximum excursions of the QRS group, there are found to be twenty-two such records. Sixteen of these patients (73 per cent) suffered from the anginal syndrome, and of the other six patients all but one had a diagnosis which might well have involved coronary narrowing. These other diagnoses were: three with myocardial fibrosis and congestive failure, and one each with syphilitic aortitis, rheumatic aortic insufficiency and neurocirculatory asthenia. This large Q-3 is evidently closely associated with any pathologic changes that involve coronary narrowing, and it would seem that the larger the Q wave in relation to the voltage of QRS, the closer is this association.

Parkinson and Bedford [4] noted the large Q-3 in a group of patients whom they considered to have had a thrombosis of a coronary branch. It occurred in nine, or 31 per cent, of the twenty-nine patients whose records they published, a figure that is comparable to the 27 per cent (eight times in thirty cases of anginal syndrome) which I found in a slightly different selection of cases. Three of their records showed a small Q-3, and two cases which at first showed a large Q-3 came to show a small one as time and healing progressed (figs. 18 and 19 in their article).

The change from a large to a small Q-3 was also observed in one of my cases (fig. 4). A boy, aged 9, had had two attacks of rheumatic cardiac involvement during the previous three years. He was not seriously ill with either of these, but the second attack lasted for nine months, was accompanied by mild chorea and left him with mitral insufficiency and slight cardiac enlargement. The first record, *A*, was taken five months after the end of the second attack, and the second, *B*, after a further interval of twenty months, during which he was well and gained weight and strength. These cases are of great interest because of their indication that a record with a small Q-3 may at times represent the healing of a process which might previously have given a record with a large Q-3. On the other hand, records with normal or left axis deviation and a small Q-3 are frequently obtained from hearts that are undoubtedly normal, so that such records cannot by any means be considered as having a pathologic significance.

Another fact has been observed that cannot be without significance in understanding the records here described. In a series of twenty-three cases of rheumatic pericarditis which were collected by Dr. Daniel Porte

4. Parkinson, J., and Bedford, D. E.: Successive Changes in the Electrocardiogram After Cardiac Infarction (Coronary Thrombosis), Heart **14**:195, 1928.

from the New York Hospital records, six cases have been found with records of this peculiar type with a large Q-3. It appears that there is something about the rheumatic invasion of the heart, perhaps especially about rheumatic pericarditis, that gives rise to a QRS group of this sort.

Certain other features related to this peculiarity of Q-3 have an important bearing on attempts to understand its meaning. A QRS in lead 3 like that seen in figure 3 *A* with a large R and small Q is a common feature of records from normal hearts, and especially of those showing right axis deviation of QRS. It occurred twenty-two times in the normal series of Lewis and Gilder, many of which indicated right axis deviation,

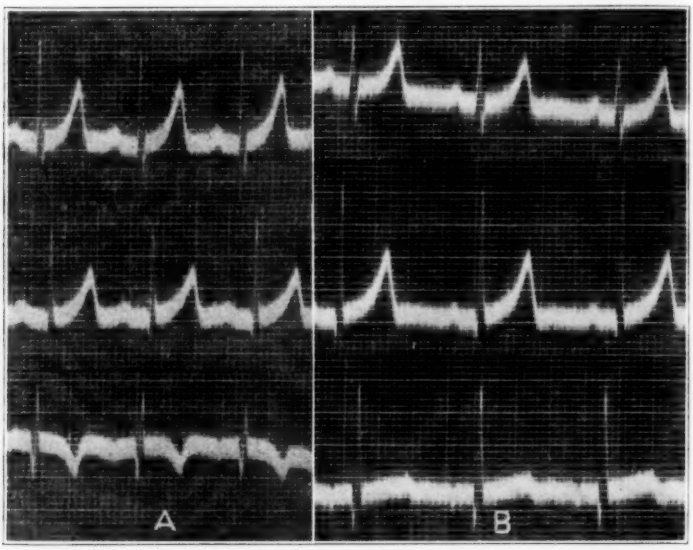

Fig. 4.—Two records of the same patient: *A*, large Q-3, more than 25 per cent of R-1; *B*, record taken eighteen months later showing a small Q-3, less than 25 per cent of R-2.

and twenty-nine times in a series of fifty of my records showing right axis deviation. This would suggest that Q-3 is related to the activity of the right ventricle. The Q wave of lead 3 has been related to the activity of the right ventricle by Lewis on other grounds.[5]

Lewis also demonstrated that when the ventricular systole starts in the right ventricle, as it does after section of the left branch of the A-V bundle, or with a right ventricular premature beat, the first portion of

5. Lewis, T.: The Spread of the Excitatory Process in the Vertebrate Heart, Phil. Tr. Roy. Soc. London **207**:221 (March) 1916.

the QRS group is inscribed by electrical currents the successive units (vectors) of which rotate progressively in a clockwise direction.[5] The QRS group of records obtained from hearts with preponderant hypertrophy of the right ventricle (curves with right axis deviation of QRS) also shows a progressive clockwise rotation of the successive electrical forces (vectors) of which it is composed.[6] Curves of a contraction starting in the left ventricle, however, and curves with left axis deviation of QRS show a counter-clockwise rotation of the electrical vectors of QRS.

As has been noted, the records with a large Q-3 here under discussion show left axis deviation, as a rule, with R-1 larger than R-2, though in

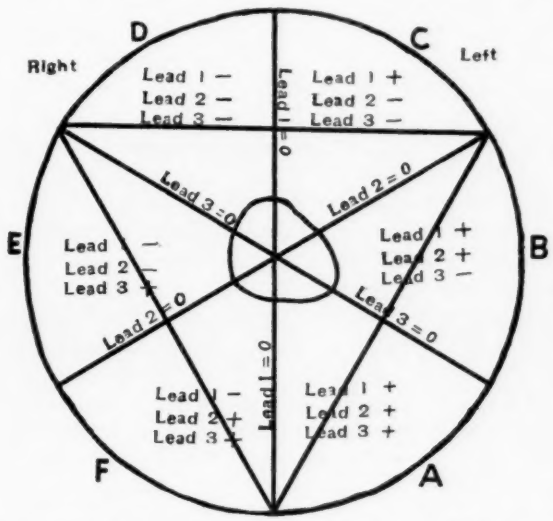

Fig. 5.—Diagram, showing direction of the deflections in the three leads resulting from vectors with various directions (after Pardee: Clinical Aspects of the Electrocardiogram).

some records the electrical axis is in the normal direction with R-2 larger than R-1 or R-3. When QRS shows left axis deviation, lead 3 does not have an R wave followed by an S, as is usual for records with left axis deviation. Instead of this, the downward deflection is a Q wave, followed by an R, and as a result the electrical currents (vectors) developed during the QRS group do not progress in a counter-clockwise direction as is usual for records with left axis deviation. The records with a large Q-3 are found to have a clockwise rotation of the vectors of QRS, no matter whether left axis deviation is present or the axis of QRS is in the normal direction. This will appear from the following.

If Q is present in all three leads the initial vectors of QRS will lie in sector D of figure 5, for the deflections will be lead 1—, lead 2—, lead 3—. Usually Q is found only in leads 2 and 3, in which case the initial deflection will be some portion of the R wave in lead 1, and some portion of Q in leads 2 and 3, so that the deflections will be lead 1 +, lead 2—, lead 3—, and the vectors will lie in sector *C*.

One comes now to a latter portion of the R in lead 1, approximately the peak. This will correspond approximately to the peak of the R wave in lead 2 and to a later portion of the Q wave in lead 3 or to an R wave in lead 3, according to the relative height of the R waves in lead 1 and in lead 2. If R-2 is greater than R-1, there must be a positive value in lead 3 which will lie in the R wave. If R-1 is greater than R-2, the corresponding value would be negative (downward) in lead 3 which will be a portion of the Q wave. If the deflections are lead 1 +, lead 2 +, lead 3—, the vector lies in sector *B* of the figure, or if lead 1 +, lead 2 +, lead 3 +, in sector *A*. This portion of the QRS group usually involves the peak of R-1 or of Q-3 and usually develops the largest voltage of the QRS group. In some records Q is found only in lead 3, so that the initial deflections are R in lead 1, R in lead 2, and Q in lead 3; the values are +, +, — and the vectors lie in sector *B* of the figure. In such records the initial deflections usually develop the largest voltage of QRS.

The last portion of QRS usually has an S in lead 1, less often an S in lead 2, and always an R in lead 3. The deflections accordingly are either — — + or — + +, and this places the vectors in sector *E* or *F* of the figure. Occasionally there is no S in lead 1 or lead 2, and such records have the last deflections upward (+) in each lead, so that the vectors lie in sector *A*. In records with a QRS of this type the largest deflection of QRS lies in this sector. It will be seen, then, that in the QRS group of records with a large Q-3 the currents may develop in various ways. The vectors may be first in sector *B* and then in *F*, or first in *C*, then in *B* or *A* and then in F or E, or first in B and then in F or E. Two things are always observed: (1) The direction of rotation of the vectors is clockwise, and (2) the vectors of the largest voltage of QRS (the electrical axis of QRS) will usually lie in sector *B*, though sometimes in sector A, indicating left axis deviation in the first case and normal direction of the electrical axis in the second. This clockwise rotation of the vectors suggests a predominance of the effect of the right ventricle during the spreading of the contraction, even though right axis deviation of QRS is not present.

The inversion of T-3 is another right ventricular effect. It is always present when the contraction starts in the right ventricle.[5] With or without inversion of T-2 it is very common (25 per cent) in records showing

right axis deviation of QRS.[6] The inversion of T-1 and T-2, on the other hand, is never found when the contraction starts in the right ventricle [5] and is very rare (3 per cent) in records showing right axis deviation.[6] In this series of thirty-three records with a large Q-3, twelve records (28 per cent) had an upward T in all leads, five records (12 per cent) showed an inversion of T-1, with or without T-2, and twenty-six records (60 per cent) showed an inversion of T-3 or of T-2 and T-3.

It must be significant that right ventricular effects are suggested by the clockwise rotation of the vectors of QRS in all of the records with a large Q-3, and that in two thirds of them the T wave is affected as with right ventricular premature beats and with preponderant hypertrophy of the right ventricle. It is suggested that these features indicate a predominance of the right ventricle during the spreading of the contraction in these hearts, in spite of their left or normal axis of QRS, and that the reason for this peculiarity may lie in a particular sort of damage to the left ventricular muscle. From the clinical studies shown in the table it appears that narrowing of a branch or branches of the left coronary system is the commonest causes of this left ventricular damage. The finding of a large Q-3 in patients with active rheumatic myocardial disease suggests disproportionate damage to the left ventricular muscle by the rheumatic process. In the patients with hypertension and cardiac arhythmia there may possibly have been a complicating coronary arteriosclerosis affecting the myocardium of the left ventricle with the large Q-3 as its sole manifestation. The explanation of this feature in the records from the four supposedly normal hearts in this series lies possibly in an incorrect diagnosis of normal heart, for two of these hearts were shown to be definitely enlarged by the orthodiagraphic tracing. It is likely that a better understanding of the causes of this wave would afford an explanation that would apply to the normal hearts as well as to the abnormal ones. Possibly an occasional cause in normal hearts may lie in peculiar variations in the branching of the A-V bundle.

It is important to note the effect of deep inspiration on the waves of a QRS group of this type. Figure 6 shows lead 3 of four patients with this peculiar wave. In the upper three records forced inspiration was present at the left end and normal expiration at the right end. It will be observed that with inspiration the Q becomes smaller and the R larger, so the form shown could not be considered abnormal. Though the left axis deviation is abolished and Q-3 made smaller, yet clockwise rotation of the vectors of QRS still remains. In this connection also it is important to note that the case (accompanying table) with normal heart and pregnancy failed to show a large Q-3 in a record taken ten

6. Pardee, H. E. B.: Clinical Aspects of the Electrocardiogram, ed. 2, Paul B. Hoeber, Inc., 1928, pp. 42 and 53.

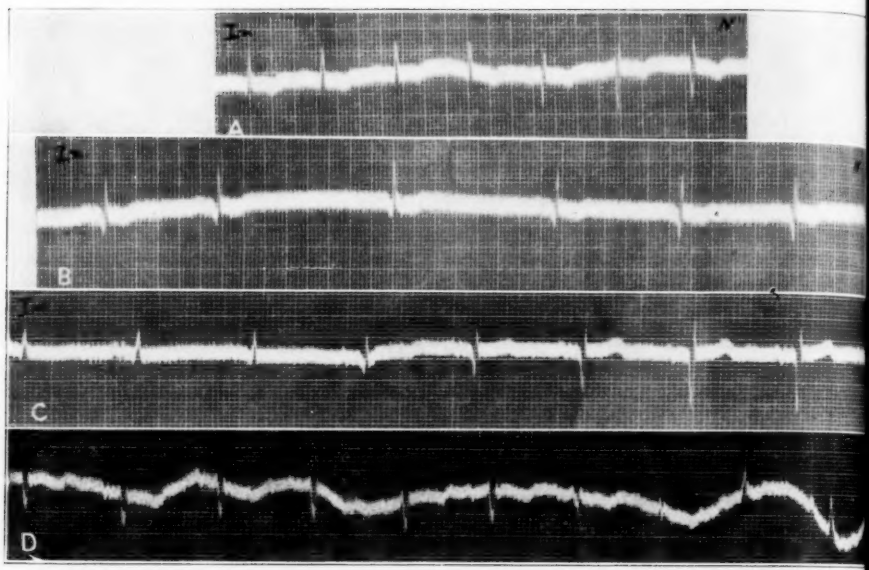

Fig. 6.—*A*, *B*, *C*, the third lead of three patients; full inspiration is present at the left, and normal expiration at the right; *D*, the third lead of the patient whose records appear in figure 7; forced expiration was present at the left, and forced inspiration at the right.

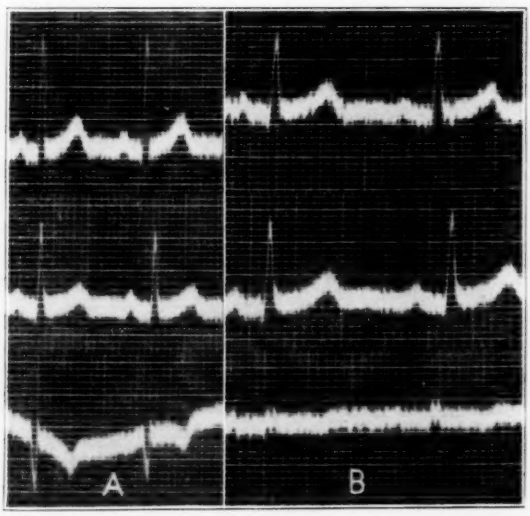

Fig. 7.—*A*, record with a large Q-3 obtained from a patient with a normal heart during the eighth month of pregnancy; *B*, record of the same patient eleven months after delivery.

months after delivery. Figure 7 shows the record taken during pregnancy which is included in this series, and also the record taken after delivery. The long strip, figure 6 *D*, is lead 3 of this pateint taken on the last occasion, forced expiration being present at the left end of the record and full inspiration at its right end. The change in the QRS during the inspiratory process is striking, and illustrates well the importance of the position of the heart within the chest. The first heart cycle of this record shows a large Q and a small R much like the lead 3 of the record taken during pregnancy. The next five cycles show a QRS of the M type; the seventh cycle has a notched QRS; the eighth closely resembles that of lead 3 taken after delivery (fig. 7 *B*), while the last two cycles show nothing but an R wave—no downward deflection. Changes in the position of the heart and diaphragm are known to cause radical changes in QRS and these changes in position appear to bear some special relation to the finding of a large Q-3 in records from certain normal hearts. A high position of the diaphragm seems to favor the appearance of a large Q-3, while a low position is unfavorable. Each of the two patients in Cohn's normal series who showed a large Q-3 had hearts that were shown by roentgenograms to be more horizontal than the average.

SUMMARY

Attention is directed to the occurrence of records showing left axis deviation of QRS or a normal electrical axis, combined with a large Q wave in lead 3, one that is 25 per cent or more of the largest deflection of QRS in whichever lead this may occur.

The majority of such records are obtained from patients with the anginal syndrome, but certain patients with myocardial fibrosis and congestive failure, certain patients with rheumatic heart disease, especially with pericarditis, and a few with hypertension will give such records. Certain patients who have cardiac symptoms but no definite evidence of cardiac disease have been found to show this large Q-3, and rarely (twice in 277 cases) such records are obtained from apparently normal hearts.

These records show a clockwise rotation of the vectors of the QRS group, and frequently there is an inversion of T-3 or of T-2 and T-3. Both of these features depend on right ventricular activity, and it is suggested that the finding of a large Q-3 indicates disease of the left ventricle, so that the right ventricle predominates during the spreading of the contraction in spite of the left axis deviation or normal axis direction of QRS. The effect of diaphragmatic movements on the large Q-3 is noted, and it is suggested that the occasional finding of a large Q-3 in normal hearts may be due to an unusual distribution of the branches of the A-V bundle and that a high position of the diaphragm may be a contributory factor.

MODIFICATION OF THE DEXTROSE TOLERANCE TEST AS AN INDEX OF METABOLIC ACTIVITY OF THE LIVER*

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Attempts to utilize disturbances of carbohydrate metabolism for a diagnosis of functional impairment of the liver are not new. But the levulose and galactose tolerance tests that are available do not give sufficiently uniform results to warrant their general adoption. The objections to these sugar tolerance tests as an indication of liver function are twofold. The first and real objection is that while sugar metabolism suffers in certain diseases of the liver, this fact is susceptible of consistent demonstration only by group averages. In individual cases of even proved diseases of the liver, the results of these tests frequently fall within the limits of normal and minimize their significance. The second objection is that other organs, such as the pancreas and the hypophysis, also play a prominent part in the metabolism of carbohydrates, and therefore any discovered abnormalities of such metabolism may not be due specifically to a deficiency of the liver.

The proposed method of testing the carbohydrate metabolism described in the following paragraphs was devised to overcome the first objection. The second objection is theoretically more difficult to overcome, but in practice with a suitable carbohydrate tolerance test at hand it should present no greater difficulties in the diagnosis of diseases of the liver than one encounters in the diagnosis of diabetes mellitus, in spite of the knowledge that other organs besides the pancreas are concerned with utilization of sugar.

The principle of the present test was evolved while we were carrying on metabolic studies on a patient having hemochromatosis with diabetes and cirrhosis of the liver, in whom fluctuations of great magnitude were observed in the blood sugar level following slight changes in the intake

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of carbohydrate or the dosage of insulin.[1] This extreme instability of the blood sugar level was ascribed to marked impairment of the glycogenetic and glycogenolytic functions of the liver, and the question arose as to whether or not a method could be found to bring out milder forms of a similar impairment in other patients suffering from hepatic disease. Such a method was found in a test whereby three functions of the liver are taxed at once, thus bringing out latent deficiencies which would escape detection if only one function were tested. This method will probably offer new possibilities in the functional study of other organs.

A review of the literature with this in mind, brought to our attention the work of Klein,[2] who observed unusually high and low blood sugar levels in patients with hepatic disease after simultaneous administration of dextrose and large amounts of water. He suggested that this might be used for functional testing of the liver. The downward fluctuation of the blood sugar level in these cases is accentuated even more by simultaneous administration of insulin.[3]

It may be mentioned here that as early as 1902, clinical disturbances of the intermediary water metabolism in hepatic disease were described and that at present the important rôle of the liver in the regulation of water metabolism is well established by numerous workers.[4]

METHOD

On the basis of this knowledge, a method was devised consisting of the administration of insulin, dextrose and water followed by observations of the blood sugar level over a period of three hours. The following schedule was found to answer our purpose and was adhered to in all reported cases: In the morning, with the patient fasting, a specimen of the blood was taken for sugar determination followed by an injection of 20 units of insulin. Twenty minutes later, 50 Gm. of dextrose dissolved in 500 cc. of water was given to the patient by mouth followed by

1. Althausen, T. L., and Kerr, W. J.: Hemochromatosis: A Report of Three Cases with Results of Insulin Therapy in One Case, Endocrinology **11**:377 (Oct.) 1927.

2. Klein, O., and Lang, E.: Ueber die Beeinflussung der alimentären Hyperglykämie bei Leberkranken durch Wasserbelastung, München. med. Wchnschr. **73**:187, 1926.

3. Klein, O.: Weitere Studien über Insulin und Wasseraushalt sowie über Insulinwirkung bei Leberkranken, Med. Klin. **21**:1116, 1925.

4. Molitor, H., and Pick, E. P.: Die Bedeutung der Leber für die Diurese, Arch. f. exper. Path. u. Pharmakol. **97**:317, 1923. Adler, A.: Der Einfluss der Leber auf die Wasserausscheidung, Klin. Wchnschr. **2**:1980, 1923. Saxl, P., and Donath, F.: Wasserhaushalt und reticuloendotheliales System, Klin. Wchnschr. **3**:1397, 1924.

1,000 cc. of water also administered by mouth. After this two samples of blood were taken at intervals of thirty minutes and two more at intervals of one hour. In cases in which insulin reactions developed during the test, the last sample of blood was taken at the time of the reaction regardless of schedule, and the patient was treated for hypoglycemia in the usual way. Previous to the test, all patients were kept on a general hospital diet for a period of at least several days.

The test has been applied to sixty-four patients with and without clinical evidence of hepatic disease. Only one of these patients experienced difficulty in taking 1.5 liters of fluids, and in no case did we meet with an insulin reaction of alarming severity.

RESULTS

In persons without evidence of hepatic disease, we observed blood sugar curves similar to that shown in the chart (solid line), which represents a composite curve of ten normal tests. It is characteristic of such curves that the blood sugar content rises during the first hour of the test and then gradually declines, until at the end of three hours it is again near the level maintained during fasting. The only difference between our composite curve and a normal blood sugar curve after the ingestion of 100 Gm. of dextrose is that in the former, the original level is reached in three hours instead of two. The same results, namely, delayed return of the blood sugar curve to normal, were obtained by Sweeney [5] in hydrated dogs subjected to the dextrose tolerance test.

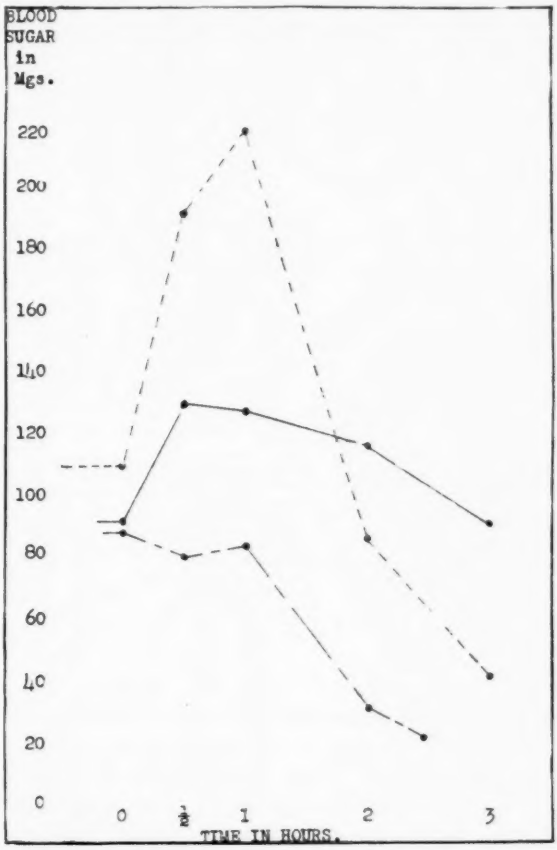
In patients with clinical evidence of hepatic insufficiency, blood sugar curves following our test were irregular, falling between the two extremes given in the same chart (broken lines). One of these curves shows marked hyperglycemia before the terminal hypoglycemia is reached and in the other there is an uninterrupted falling off of the blood sugar level during the entire duration of the test. However, all such abnormal curves have in common the eventual low blood sugar level, usually accompanied, as would be expected, by insulin reactions.

The conclusion from this was that, terminal hypoglycemia under the conditions of the test meant injury of the liver. The question immediately arose as to the dividing line between the normal and the abnormal. After clinical study of our cases and comparison with other liver function tests, principally the rose bengal dye excretion test, we decided to place it in the vicinity of 70 mg. of sugar per hundred cubic centimeters of blood. It is of interest that Hewlett [6] also gave this figure as the lowest normal.

5. Sweeney, J. S.: Dietary Factors that Influence the Dextrose Tolerance Test, Arch. Int. Med. **40**:818 (Dec.) 1927.

6. Hewlett, A. W.: Pathological Physiology of Internal Diseases, ed. 2, New York, D. Appleton & Company, 1923, p. 270.

The average lowest blood sugar level in fifty-five positive modified dextrose tolerance tests was 50 mg. The individual readings ranged from 23 to 70 mg. but only ten of these patients had a minimum blood sugar level of over 55 mg. On the other hand, in ten negative tests the average lowest blood sugar was 95 mg., with variations between 73 and



Normal and two abnormal blood sugar curves following the modified dextrose tolerance test. The solid line represents an average of ten normal tests. The regularly broken line gives our most outspoken hyperhypoglycemic curve. The irregularly broken line traces the most marked simple hypoglycemic curve in this series.

122 mg., and only four cases reached a level below 98 mg. Such a wide zone between positive and negative results is considered in most cases one of the important advantages of the modified dextrose tolerance test.

The interpretation of differences between various types of abnormal blood sugar curves, aside from the final low blood sugar level, is not clear. We do not know whether these are expressions of hepatic insufficiency of various degrees, or whether we are dealing with impairment of the entire carbohydrate metabolism-regulating function of the liver in some cases and with dysfunction of glycogenolysis only in others. We are also unable to correlate these variations with any clinical entity involving the liver.

It is of interest that in positive cases the degree of hypoglycemia toward the end of the test is little influenced by the maximum height of the blood sugar curve, as the following figures will demonstrate: In cases in which the peak of the blood sugar curve was from 140 to 221

TABLE 1.—*Diseases of the Liver*

		Modified Dextrose Tolerance Test				Rose Bengal Test			
Diagnosis	Number of Cases	Positive	Negative	Average in Mg.	Range of Readings in Mg.	Positive	Negative	Aver. at 16 Min. in %	Range of Readings in %
Portal cirrhosis	4	3	1*	47	41-55 (144)*	3	..	55	41-63
Toxic cirrhosis	5	1†	5	85	70-122 (52)†	4‡	..	84	83-84
Syphilitic cirrhosis	2	2	..	46	40 and 52	1	1§	..	65 and 0§
Probably early cirrhosis	6	6	..	47	35-66	6	..	49	35-77
Metastatic carcinoma of the liver	5	5	..	52	40-60	4¶	..	56	45-70
Echinococcus cyst of the liver	2	2	..	45	39 and 50	2	..	48	35 and 61
Infectious jaundice	4	3	1#	57	48-69 (224)#	4	..	58	35-87
Arsphenamine jaundice	2	2	..	49	31 and 67	2	..	62	38 and 85
Complete obstructive jaundice	1	1	..	..	23	..‖	..		

* Patient also had diabetes with blood sugar of 157 mg. during fasting.
† During the acute stage; later it became negative.
‡ Deep icterus in one case precluded the rose bengal test.
§ Atypically negative, probably due to splenomegaly.
¶ Deep icterus in one case precluded the rose bengal test.
# Patient also had diabetes with blood sugar of 162 mg. during fasting.
‖ Rose bengal test omitted due to deep jaundice.

mg., the average lowest blood sugar at the end of the test was 50.5 mg.; in cases in which the maximum blood sugar reached between 100 and 140 mg., it was 48.4 mg., and in cases in which the blood sugar curve at all times remained below 100 mg., it was 40.4 mg.

Diseases of the Liver.—Thirty-one cases of hepatic disease were studied with the results shown in table 1. It will be noticed at once that in all but one of the twelve cases of portal, syphilitic and suspected cirrhosis of the liver, the modified dextrose tolerance test gave positive results. The negative results in the single exception were due to an associated diabetes mellitus with a high blood sugar level at the beginning of the test. The significance of this will be taken up in a discussion on limitations of the present test. On the other hand, all five patients with toxic cirrhosis of the liver were proved to have normal carbohydrate metabolism during the chronic stage of their disease. One of these

patients did show positive results in the modified dextrose tolerance test, but only when seen during one of her acute attacks. These recurrent attacks in the course of toxic cirrhosis are, according to Mallory and others, in the nature of subacute yellow atrophy of the liver. The preservation of normal carbohydrate metabolism in this form of cirrhosis of the liver is explained by active regeneration of hepatic cells, which, however, in contrast to other types of cirrhosis, do not connect with bile channels and therefore are unable to partake in excretory processes.[7]

In seven patients with localized lesions of the liver due to metastatic carcinoma and to echinococcus cysts, the modified dextrose tolerance test gave uniformly positive results. Likewise, six of seven patients suffering from various types of jaundice had impaired carbohydrate metabolism

TABLE 2.—*Disease of the Hemopoietic and Lymphatic Systems*

Diagnosis	Number of Cases	Modified Dextrose Tolerance Test				Rose Bengal Test			
		Positive	Negative	Average in Mg.	Range of Readings in Mg.	Positive	Negative	Aver. at 16 Min. in %	Range of Readings in %
Myeloid leukemia (hepatomegaly in all but one case)	4	4	..	52	42-65	3	1*	60	58-63 (5)*
Lymphoid leukemia with hepatomegaly	1	1	..	..	45	1	..	..	68
Pernicious anemia with large liver	1	1	..	..	41	1	..	..	39
Banti's disease with hepatomegaly	1	1	..	..	65	1	..	..	65
Hodgkin's disease with large liver	1	1	..	..	48	1	..	..	38
Splenomegaly (marked unexplained)	1	1	..	..	47	..	1	..	30†
Polycythemia vera with hepatomegaly	1	1	..	..	33	..	1‡	..	20

* Atypically negative in patient with marked splenomegaly; only patient without enlarged liver.
† Upper limit of normal.
‡ Splenomegaly present.

regulation. The negative results in one case were brought about again by a complicating diabetes with high fasting sugar content of the blood.

Diseases of the Hemopoietic and Lymphatic Systems.—In all ten patients with various afflictions of the hemopoietic or lymphatic systems, the modified dextrose tolerance test detected abnormalities of carbohydrate metabolism (table 2). In eight of these cases, marked hepatomegaly was the chief reason for testing the function of the liver.

Miscellaneous Cases.—In this group shown in table 3 the modified dextrose tolerance test was positive in seven patients who had marked multiple chronic infections, such as pulmonary tuberculosis, gonorrheal prostatitis and chronic sinusitis in the same patient. The carbohydrate

7. The details of this process with presentation of pathologic material will be published shortly by the senior author under the title, "Functional Aspects of Regenerated Hepatic Tissue."

metabolism-regulating function of the liver was also found to be impaired in two cases of arsenical dermatitis and in one case of chronic interstitial nephritis with ascites and pleural and pericardial effusions.

The test was negative in two cases of arteriosclerosis with hypertension, and in one case each of carcinoma of the urethra and uncomplicated chronic interstitial nephritis.

Limitations of the Test.—Theoretically, one could expect any abnormalities of the carbohydrate or water metabolism other than those attributed to the liver to invalidate the modified dextrose tolerance test. With

TABLE 3.—*Miscellaneous Cases*

Diagnosis	Number of Cases	Modified Dextrose Tolerance Test				Rose Bengal Test			
		Positive	Negative	Average in Mg.	Range of Readings in Mg.	Positive	Negative	Aver. at 16 Min. in %	Range of Readings in %
Multiple chronic infections	7	7	..	42	35-60	5	1	37	10-55
Arsenic dermatitis	2	2	..	39	31 and 47	2	..	57	40 and 74
Chronic nephritis	2	1*	1	..	33 and 98	2	..	67	65 and 68
Arteriosclerosis with hypertension	2	..	2	107	106 and 108	1†	1	..	20 and 80
Carcinoma of urethra	1	..	1	..	109	1	..	..	40

* Patient had fluid in all serous cavities.
† Patient had chronic passive congestion.

TABLE 4.—*Conditions which Theoretically Should Interfere with Estimations of Carbohydrate Metabolism*

Diagnosis	Number of Cases	Modified Dextrose Tolerance Test				Rose Bengal Test			
		Positive	Negative	Average in Mg.	Range of Readings in Mg.	Positive	Negative	Aver. at 16 Min. in %	Range of Readings in %
Diabetes mellitus	2	..	2*	..	73 and 291	1	1	..	20 and 80
Thyrotoxicosis	4	4†	..	50	42-61	2	..	68	48 and 87
Myasthenia gravis	1	1‡	..	..	57	..	1	..	15
Diabetes insipidus	1	1	..	..	43	..	1	..	30§

* Patient with positive rose bengal test had a blood sugar level of 328 mg. during fasting.
† Patient also had diabetes, but blood sugar during fasting was 133 mg.
‡ Patient also had diabetes, but blood sugar during fasting was 101 mg.
§ Upper limit of normal.

this in mind we studied two common and two rare conditions involving such metabolic disturbances (table 4).

Five patients with and without evidence of hepatic disease whose conditions gave a typical diabetic curve in the ordinary dextrose tolerance test were subjected to our carbohydrate liver function test. In three of these, the blood sugar level during fasting was high at the beginning of the test. All three cases gave negative results in the face of marked rose bengal dye retention, and there was well marked clinical evidence of hepatic disease in two cases. On the other hand, of two patients with diabetes and a nearly normal blood sugar level during fasting at the

beginning of the test, one had normal and the other impaired carbohydrate metabolism regulation. In both cases there was agreement with the rose bengal test. The obvious conclusion is that diabetes per se does not interfere with the accuracy of the modified dextrose tolerance test, provided there is no marked hyperglycemia at the beginning of the test.

Four patients with marked thyroid hyperplasia with basal metabolic rates between 46 and 85 per cent plus were also subjected to the carbohydrate function test for the purpose of testing the possibility of abnormally low blood sugar levels, being due to increased oxidation of dextrose in this disease. All four patients gave positive results to our test, and although in both cases in which it was done the rose bengal test confirmed the presence of hepatic damage, it still remains to be shown that negative results to the modified dextrose tolerance test can take place in the presence of a high basal metabolism. Indirect proof in favor of this is found in the fact that, in a considerable proportion of such cases, the increased metabolism associated with hyperthyroidism does not prevent hyperglycemia with galactosuria following galactose tolerance tests. Functional impairment of the liver in severe thyrotoxicosis is probably accounted for by focal necrosis of this organ sometimes seen at postmortem examination.

In one case of myasthenia gravis, with diminished dextrose tolerance, the modified dextrose tolerance test showed positive results and the rose bengal test negative results. Disturbances of carbohydrate metabolism, according to Gibson and his associates,[8] are the rule in cases of myasthenia gravis. In addition, this condition is a disease of muscles which, as shown by Engels,[9] play an important rôle in the intermediate water metabolism. Thus we are dealing with an entity in which all metabolic functions involved in our test are affected and combine to invalidate its results.

One patient with diabetes insipidus also showed definite impairment of carbohydrate metabolism regulation, but in this case, the rose bengal test being just at the upper limit of normal made it impossible to say without study of more cases whether we were dealing with real injury to the liver or not. Theoretically, it is quite probable that the disturbances of water metabolism characteristic of diabetes insipidus, especially the frequently present unusual delay in the elimination of extra quantities of liquids, would interfere with the accuracy of the test.

8. Gibson, R. B.; Martin, F. T., and Van Rennselaer Buell, M.: A Metabolic Study of Progressive Pseudohypertrophic Muscular Dystrophy and Other Muscular Atrophies, Arch. Int. Med. **29**:82 (Jan.) 1922.

9. Engels, W.: Die Bedeutung der Gewebe als Wasserdepots, Arch. f. exper. Path. u. Pharmakol. **51**:346, 1903.

COMPARISON OF RESULTS OBTAINED WITH THE MODIFIED DEXTROSE TOLERANCE AND THE ROSE BENGAL TESTS

In all but seven of our cases the rose bengal dye liver function test was performed for comparison. In three of these cases, the presence of deep icterus made it superfluous because in any case of jaundice dye elimination is impaired at least in proportion to the depth of jaundice. We realize that since the rose bengal test is an excretion test, while the modified dextrose tolerance test is a metabolic one, agreement between the two tests cannot be expected in all instances. On the other hand, a confirmation of the results of the modified dextrose tolerance test, which is in the experimental stage, by an established test like that of rose bengal dye elimination was of great value in this work, especially in cases in which, from the clinical point of view, there were no definite reasons to suspect damage to the liver.

In comparing the results of these two liver function tests, we must exclude cases of: (1) toxic cirrhosis (in which the very contrast between the results of the two tests constitutes a valuable diagnostic aid); (2) diabetes mellitus with high blood sugar level during fasting; (3) myasthenia gravis and diabetes insipidus, and (4) splenomegaly which may give false negative results with the rose bengal test, apparently because of overdevelopment of the reticulo-endothelial system of the spleen which takes an important part in the disposal of dyes used for the functional testing of the liver.[10]

It exceeded our most optimistic expectations to find that after eliminating cases in which inaccuracy of one or the other of the two tests can be predicted *a priori*, there were only four cases in which the results of these liver function tests did not agree. In three of these with diagnoses of chronic interstitial nephritis, arteriosclerosis with hypertension, and carcinoma of the urethra, the modified dextrose tolerance test gave negative results. In the fourth, a case of multiple chronic infections, the rose bengal elimination was normal. Translated into percentages, this means 91 per cent agreement between the two tests as to presence or absence of hepatic damage in all our cases. Moreover, in patients with diseases of the liver and of the hemopoietic and lymphatic organs, comprising forty-one cases, there was complete agreement in the outcome of the two tests.

PHYSIOLOGIC BASIS OF THE MODIFIED DEXTROSE TOLERANCE TEST

Attempts at explanation of the mechanism involved in the production of hypoglycemia under the conditions of our test in cases of hepatic disease, at the present stage of our knowledge, must of necessity carry

10. Schellong: Funktionsprüfung der Leber oder des reticuloendothelialen Systems mit Farbstoffen, Med. Klin. **22**:1711, 1926.

us into the field of speculation. However, we believe that we are in the possession of sufficient evidence to point out a probable theory.

This theory has as its basis the assumption suggested by Klein [3] that in diseases of the liver there is slowing of metabolic activity on the part of its cells. Insulin which is given at the beginning of the test apparently promotes oxidation of sugar in the tissues and at the same time tends to inhibit glycolysis, in this way bringing about lowering of the blood sugar level. In addition, the ingestion of a large amount of water, which may or may not gain premature admission to the general blood stream in cases of hepatic injury, diminishes the blood sugar content even further. A parallel to this is described by Epstein,[11] in cases in which diabetic hyperglycemia is masked by an increase in blood volume. In normal persons the dextrose ingested during the test seems to be polymerized to glycogen and in turn released as dextrose with sufficient rapidity to counteract impending hypoglycemia, whereas in patients with hepatic disease this process is sufficiently slowed to permit of reaching abnormally low blood sugar levels.

The part played by the glycogen reserve of the liver at the time of the test is of great importance. The connection between the glycogen content of the liver and its functional activity is well known. To mention only two examples, a high glycogen conetnt of this organ brings about faster assimilation of carbohydrates [12] and also increases the detoxifying power of the liver.[13] We know that in some diseases of the liver its glycogen content is markedly depleted,[14] and therefore the possibility that this may be responsible for hypoglycemia under the conditions of the test must be considered.

On the other hand, without denying that the glycogen content of the liver is playing some part in the outcome of the modified dextrose tolerance test, it is safe to assert that it is not the only factor involved because, by giving insulin and water without the dextrose we can produce hypoglycemia in normal persons in whom the addition of 50 Gm. of dextrose is capable of preventing an undue lowering of the blood sugar level.

Since this question is important not only from the diagnostic but also from the therapeutic point of view, because the answer to it would throw

11. Epstein, A. A.: The Influence of Renal Function on Hyperglycemia and Glycosuria in Diabetes Mellitus, Am. J. M. Sc. **154**:103, 1917.

12. Staub, H.: Untersuchungen über den Zuckerstowechsel des Menschen, Ztschr. f. klin. Med. **93**:89, 1922.

13. Opie, E. L., and Alford, L. B.: Diet and the Hepatic Lesions of Chloroform, Phosphorus or Alcohol, J. Exper. Med. **21**:1, 1915.

14. Ravdin, I. S.: Some Aspects of Carbohydrate Metabolism in Hepatic Disease, J. A. M. A. **93**:1193 (Oct. 19) 1929. Umber, F.: Akute und subacute Leberatrophie, Klin. Wchnschr. **1**:1585, 1922.

more light on the combined dextrose and insulin treatment of diseases of the liver, we have plans under way to test this combined treatment by animal experimentation.

Other possible theories which were given by Klein,[15] who did much fundamental work in this field, seem less probable to the authors but deserve mention. One of these, based on the work of Lesser,[16] accounts for the hypoglycemia by an undue hydration of the liver with consequent swelling resulting in a separation of the diastatic ferment from the glycogen within the individual hepatic cells.

Another theory advanced by Klein is founded on the researches of Haüsler and Loewi,[17] who claimed to have isolated a substance "glykamine" which is antagonistic to insulin. In health, these two substances are said to be in equilibrium, but in diseases of the liver the dextrose releasing "glykamine" is reduced in amount with a consequent tendency toward latent hypoglycemia, which is brought out under conditions of the modified dextrose tolerance test.

Before conclusion of this paper, it seems timely to remove any remaining doubts that a function of the liver is on trial in the modified tolerance dextrose test. We know that by our method glycogenolysis on the part of the liver is being tested, in the first place, because Simpson and Macleod [18] showed that liver glycogen is the only source of blood sugar; in the second place, because Villa [19] found at the height of insulin hypoglycemia the lowest sugar concentration to be not in the peripheral blood but in that of the hepatic veins. We also have indications that glycogenesis is involved in this test because hypoglycemia is produced in normal persons if the administration of dextrose is omitted from the test.

The question may be asked whether the diet preceding the modified dextrose tolerance test can influence its outcome, since Sweeney [5] demonstrated that antecedent diets of an extreme nature definitely alter the carbohydrate tolerance of persons. The answer to this is given by later

15. Klein, O., and Holzer, H.: Beobachtungen über Insulinhypoglykämie, Insulinshock und Insulinleukocytose beim Menschen, Ztschr. f. klin. Med. **106**:360, 1927.

16. Lesser, E. J.: Die Wechselbeziehung zwischen Glykogen und Traubenzucker in der Leberzelle und ihre Bedeutung für die Lehre vom Pankreasdiabetes, Ergb. d. inn. Med. u. Kinderh. **16**:279, 1918.

17. Haüsler, H., and Loewi, O.: Untersuchungen über Diabetes und Insulinwirkung, Arch. f. exper. Pathol. u. Pharmakol. **123**:56, 1927.

18. Simpson, W. W., and Macleod, J. J. R.: The Immediate Products of Post-Mortem Glycogenolysis in Mammalian Muscle and Liver, J. Physiol. **64**:255 (Dec.) 1927.

19. Villa, L.: Die Rolle der Leber während der Insulinhypoglykämie, Klin. Wchnschr. **4**:551, 1925.

work of the same author [20] showing that dextrose tolerance was not appreciably influenced when antecedent standardized diets were compared with uncontrolled general diets. As already mentioned, all tests in the present series were carried out on the stomach during fasting, after the patients had been on a general hospital diet for at least several days.

SUMMARY

Evidence is presented to show the value of the modified dextrose tolerance test as a metabolic liver function test.

The test possesses the following advantages: (*a*) It is simple and requires no special equipment. (*b*) It is harmless to the patient. (*c*) Unlike the levulose and galactose tolerance tests, it is reliable not only in disease groups but also in individual cases. (*d*) It brings out latent functional impairment of the liver and permits one to follow the progress of the disease. (*e*) It checks well with the results of the rose bengal test. (*f*) As a metabolic test, it results are probably independent of biliary obstruction. (*g*) In combination with a dye excretion test, it offers valuable diagnostic aid in toxic cirrhosis of the liver.

The chief limitation of the modified dextrose tolerance test is its inaccuracy in cases of diabetes mellitus with marked morning hyperglycemia.

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20. Sweeney, J. S.: A Comparison of the Effects of General Diets and of Standardized Diets on Tolerance for Dextrose, Arch. Int. Med. **42**:872 (Dec.) 1928.

RENAL DAMAGE FOLLOWING ADMINISTRATION OF MERBAPHEN (NOVASUROL)

REPORT OF NINE CASES *

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Merbaphen (novasurol) is accepted as a useful diuretic in many cases of edema due to congestive heart failure. Attention has been called by Marvin [1] and others to its immediate toxic effect in some cases. That there may be damage to the renal epithelium for as long as four weeks after the last injection of merbaphen was mentioned by Redlich.[2] He reported an instance of damage to the tubular epithelium of the kidney in a patient with tuberculosis who died four weeks after an injection of 1 cc. of the drug. Saxl [3] immediately claimed that the damage to the kidney could not have been due to the merbaphen because of the period of time that had elapsed since its use. He said, moreover, that even if the mercury were responsible for the lesions, it was given under conditions that clearly contraindicated its use. The question of the toxicity of merbaphen raised by Redlich and Marvin has engaged my interest because of certain experiences which I have had.

Among 741 necropsies performed between Jan. 1, 1926, and Dec. 31, 1928, nine instances were found in which the patients received merbaphen during the clinical course of their illness. In three of these cases, extensive necrosis of the renal epithelium was observed. Attention is drawn to the fact that in case 1 merbaphen was given under circumstances adverse to its use, while in cases 2 and 8, conditions which may be considered as counterindications were also present. In the case reports given herein only the points in the clinical history and necropsy observations which seem particularly relevant are summarized.

REPORT OF CASES

CASE 1.—*Clinical History.*—M. S., a negress, aged 53, a cook, had dizziness, blurring of vision, dyspnea and edema of the ankles of one year's duration. Dur-

* Submitted for publication, Feb. 28, 1930.
* From the Department of Pathology, Yale University School of Medicine.

1. Marvin, H. M.: Merbaphen (Novasurol) as a Diuretic in Congestive Heart Failure, J. A. M. A. **87**:1016 (Sept. 25) 1926.

2. Redlich, Fritz: Letale Quicksilberintoxikation nach einmaliger Novasurolinjektion, Wien. klin. Wchnschr. **38**:350, 1925.

3. Saxl, Paul: Letale Quicksilberintoxikation nach einmaliger Novasurolinjektion, Wien. klin. Wchnschr. **38**:437, 1925.

ing this time she was under constant medical attention, and her blood pressure was known to have been elevated. Her condition improved whenever she was confined to bed. On admission examination showed the temperature to be 98.4 F., the pulse rate 120 per minute, the respirations 22 per minute and the blood pressure 153 systolic and 110 diastolic. The patient was dyspneic and edematous. The heart was enlarged. A systolic murmur, heard over the precordium, was loudest in the apical region. A diastolic murmur was audible over the lower part of the sternum. The second aortic sound was accentuated. Moist, crackling râles were present over the bases of both lungs. The abdomen was distended, and the liver was enlarged. An ophthalmoscopic examination showed the right disk to be partially obliterated by a large, white patch. An electrocardiogram revealed a left bundle branch block. The specific gravity of the urine varied between 1.010 and 1.014. During the first week in the hospital, with the patient on digitalis therapy, the output of urine was less than one third of the fluid intake. Six days after admission, the patient was given 1 cc. of merbaphen, and three days later an additional 2 cc. The second dose produced a slight diuresis. Repeated doses of theophylline, however, produced a diuresis which resulted in the loss of 22 pounds (10 Kg.) in four days. Following this treatment the condition of the patient gradually improved until four days before death, when she complained of pain in the right side and began to cough up a bloody sputum. Dulness was found over the base of the right lung, and numerous hemorrhages were seen in the retina. The temperature rose to 101 F., the leukocyte count to 25,000 cells. Following the rise in temperature, an additional dose of merbaphen resulted in bloody diarrhea. The patient grew rapidly worse, and died thirty-one days after admission to the hospital and three days after the last administration of merbaphen.

The clinical diagnosis was arteriosclerosis with hypertension and heart disease, congestive heart failure, intraventricular block and auriculoventricular block, acute mercury and digitalis poisoning and infarcts of the lungs.

Pathologic Report.—At necropsy, performed twenty-two hours after death, the kidneys were found not to be enlarged and to present only a few arteriosclerotic scars. They were bright red and were slightly swollen. Nothing else of note was observed on gross examination. Microscopically, the kidney showed the architecture to be well preserved and well stained, except for the convoluted tubules. The cytoplasm of these cells was extremely granular, and the nuclei were either pyknotic or missing. No calcification of these cells and no nuclei undergoing mitotic division were observed. The mucosa of the small intestine was present throughout. The mucosa of the large intestine was covered with a dull, greenish-black, opaque membrane. When the membrane was removed, the mucosa was found to be pierced by numerous indented ulcers, between which the mucosa appeared bright red and hemorrhagic. Microscopic preparations of the ileum showed only a few small areas of necrosis in the mucosa, while preparations of the colon showed the mucosa to be necrotic in some places and to contain hemorrhagic areas in others. The submucosa and muscularis showed evidence of edema and a diffuse polymorphonuclear leukocytic infiltration.

The complete anatomic diagnosis was:

Primary: (*a*) Necrosis and calcification of the myocardium; cardiac hypertrophy and dilatation; chronic passive congestion of the viscera; general anasarca; mural cardiac ventricular and auricular thrombi; pulmonary and splenic infarcts; necrotizing colitis,[4] and epithelial necrosis of the kidney.[4]

4. Mercury poisoning from merbaphen.

(*b*) Diffuse pneumonia; serofibrinous pleurisy; arteritis of the pulmonary artery, and cloudy swelling of the viscera.

Subsidiary: Adenoma of the thyroid, peritoneal adhesions and uterine myomas.

CASE 2.—*Clinical History.*—T. C., a white man, aged 23, a cable operator, was admitted to the New Haven Hospital because of swelling of the legs and ankles. The present illness dated from an attack of pleurisy two years before admission, from which the patient recovered and returned to work. Five months later he had an acute infection of the upper respiratory tract; heart disease was diagnosed, and he was placed on digitalis therapy. Three months later the abdomen began to swell, and a large amount of fluid was removed. Following this treatment the scrotum and legs began to swell. He also began to be troubled with hemorrhoids. The lower part of the right side of the chest was flat to percussion, and breath sounds were absent. The abdomen was distended with fluid, and the liver was felt 8 cm. below the costal margin. The lower extremities were markedly edematous. During the patient's course in the hospital the temperature varied between 98 and 102 F., and the pulse rate between 80 and 130 beats per minute. He was given 1 cc. of merbaphen on the third day after admission and 2 cc. additional two days later. This last dose produced a marked diuresis. The specific gravity of the urine varied between 1.010 and 1.014. Twenty-five days before death there was, apparently, a thrombosis of the popliteal vein. The patient died quietly thirty-five days after admission to the hospital and thirty days after the last administration of merbaphen.

The clinical diagnosis was undetermined.

Pathologic Report.—Necropsy, performed seven and one-half hours after death, showed a chronic fibrous and proliferative pulmonary tuberculosis. The kidneys were found to be of normal size. They were similar in every respect to those described for case 1. The intestines were remarkable only for some congestion of the blood vessels.

The complete anatomic diagnosis was:

Primary: (*a*) Chronic fibrous and proliferative pulmonary tuberculosis (bilateral superior lobe); pulmonary emphysema; bronchiolectasis (bilateral superior lobe); tuberculous myocarditis and pericarditis with extensive adhesions; cardiac hypertrophy and dilatation; chronic passive congestion of viscera; ascites; tuberculosis of the liver; organizing thrombus in the right auricular appendage, and thrombophlebitis of the popliteal veins (bilateral).

(*b*) Epithelial necrosis of the kidney.

Subsidiary: Recent infarct of the kidney.

CASE 3.—*Clinical History.*—K. L., a white man, aged 51, an advertising agent, was admitted to the New Haven Hospital with the complaint of shortness of breath and orthopnea of eighteen months' duration. A diagnosis of arteriosclerotic heart disease with congestive heart failure was made. His condition improved slightly with rest in bed, and he left the hospital only to be forced to return in a few days. Edema gradually increased, and in an attempt to produce diuresis 0.75 cc. of merbaphen was administered, and five and ten days later additional doses of 2 cc. were given. The last two doses produced a slight diuresis. Two weeks later the patient was admitted to the hospital for the third time. The temperature was 98.4 F., the pulse rate was 82 and the respirations 32 per minute. The blood pressure was 115 systolic and 90 diastolic. From 20 to 40 per cent phenolsulphonphthalein was excreted in two hours and ten minutes. The specific gravity of the urine varied between 1.028 and 1.030. The results of other labora-

tory tests were essentially negative. The condition of the patient grew progressively worse, edema gradually increased and death occurred eighteen days after the patient's last admission to the hospital and thirty-five days after the last dose of merbaphen.

The clinical diagnosis was arteriosclerotic heart disease with congestive heart failure and paroxysmal auricular tachycardia.

Pathologic Report.—Necropsy, performed three hours after death, showed the kidneys to be approximately normal in size. They were dark red and slightly swollen but showed nothing else of note grossly. Microscopic preparations of the kidney showed numerous vacuoles in the glomerular tufts. The remainder of the kidney, except for the cells forming the convoluted tubules, was well stained and preserved. The cytoplasm of the convoluted tubules was pale pink and granular. Many of the cells had desquamated into the lumen of the tubules. The nuclei of these cells were either missing or pyknotic, although there were several mitotic figures seen as evidence of regeneration. The intestines showed nothing of note either grossly or microscopically.

The complete anatomic diagnosis was:

Primary: (*a*) Fibrosis of the myocardium; cardiac hypertrophy and dilatation, chronic passive congestion of the viscera; ascites and anasarca; pulmonary atelectasis (left), and pulmonary emphysema (right).

Subsidiary: Epithelial necrosis of the kidney;[4] fibrous adhesions of the pleura (right); fibrous adhesions of the cecum to the abdominal wall, and cyst of the kidney (left).

CASE 4.—*Clinical History.*—S. N., a white man, aged 52, a baker, was first admitted to the New Haven Hospital with the complaint of dyspnea and swelling of the feet. The symptoms began two years before admission, when he was forced to give up work. His condition improved with rest in bed, and he was discharged from the hospital. Six months later he was readmitted, following an infection of the upper respiratory tract, complaining again of edema and dyspnea. After rest and digitalis therapy, he was discharged as improved. He discontinued the use of digitalis at home, and was readmitted four months after his second discharge from the hospital. At this time the temperature was 98.6 F., the respirations were 32 per minute, the pulse rate 80 per minute and the blood pressure 122 systolic and 105 diastolic. The patient was edematous. The liver extended to the umbilicus. The heart was enlarged, and bubbling râles were heard over the bases of both lungs. The amount of phenolsulphonphthalein excreted in two hours and ten minutes was 65 per cent, and the specific gravity of the urine varied between 1.016 and 1.030. In an attempt to relieve the edema, 1.2 cc. of merbaphen was given, and three, nine and twenty-three days later, additional doses of 2 cc. each were administered. All of these resulted in an excellent diuresis. The patient's general condition grew steadily worse, and death occurred on the forty-fourth day after his last admission and twenty days after the last dose of merbaphen.

The clinical diagnosis was arteriosclerotic heart disease, aortic stenosis and congestive heart failure.

Pathologic Report.—Necropsy, performed two hours after death, showed the kidneys to be dark red and slightly swollen but of about average size. Microscopic preparations of the kidney showed the tissue to be well stained and preserved except for the cells forming the convoluted tubules. The cytoplasm of these cells was slightly granular. The cells in some instances were swollen to such an extent that the lumen was completely obliterated, while in other places the lumina of the

tubules contained a large amount of pink-staining, homogeneous material. The nuclei of some of these cells were vesicular. The complete anatomic diagnosis was:

Primary: (*a*) Calcification and stenosis of the aortic valve; cardiac hypertrophy; passive congestion of the viscera; edema of the extremities; hydrothorax (bilateral); mural thrombi of the left auricular appendage, auricle and left ventricle, with focal myocardial fibrosis; acute aortic endocarditis; thrombus in the abdominal aorta, and thrombi of the pulmonary vessels with pulmonary infarct.

Subsidiary: Left varicocele.

Case 5.—*Clinical History.*—N. D., a white woman, aged 51, had always been subject to nose bleed. She first noticed swelling of the ankles, palpitation and shortness of breath on exertion seven years before death. These symptoms became gradually worse, and two and one-half years later she was admitted to the New Haven Hospital where the diagnosis of hemangiomas of the skin and mucous membranes, rheumatic heart disease, mitral stenosis and regurgitation, aortic and tricuspid regurgitation and congestive heart failure was made. The patient remained in the hospital for about a month and was discharged greatly improved. For two years she remained fairly well. During the last two and one-half years of her life she was admitted to the hospital three times because of edema. During this time she was given three 1 cc. doses and eight 2 cc. doses of merbaphen, all of which resulted in a marked diuresis. The last dose was given seven weeks prior to death. Death occurred after an infection of the upper respiratory tract. The specific gravity of the urine varied between 1.010 and 1.024.

The clinical diagnosis was hemangiomas of the skin and mucous membranes; rheumatic heart disease; mitral stenosis and regurgitation; aortic and tricuspid regurgitation; congestive heart failure, and auricular fibrillation.

Pathologic Report.—Necropsy, performed eighteen hours after death, showed large, pale, slightly swollen kidneys. Numerous cortical scars were seen. One kidney contained a small abscess.

Microscopic preparations showed the kidneys to be well stained and the architecture well preserved. The cells lining the convoluted tubules were low, their cytoplasm was granular and the nuclei were deep-staining. One section showed the abscess described in the gross.

The complete anatomic diagnosis was:

Primary: (*a*) Healed mitral, tricuspid and aortic endocarditis; cardiac hypertrophy and dilatation (involving particularly the right ventricle); pulmonary emphysema; pulmonary fibrosis; passive congestion of the viscera, and fibrosis of the liver; ascites.

Subsidiary: Focal pneumonia; scars of the kidney; abscess of the kidney (right); adenoma of the thyroid, and fibromas of the uterus.

Case 6.—*Clinical History.*—T. B., a farm laborer, aged 53, had had dyspnea on exertion and orthopnea for nine months. These symptoms improved with rest, but he had been unable to work during that period. The orthopnea finally became so marked that he was sent to the hospital. On admission, examination showed the temperature to be 97.5 F., respirations 20 per minute, the radial pulse rate 40, the apical pulse rate 80 and the blood pressure 145 systolic and 110 diastolic. The patient was markedly cyanotic, and there was some edema of the extremities. He improved with rest in bed and digitalis therapy but continued to have some edema. He was given three 2 cc. doses of merbaphen seventy-seven, eleven and six days before death. An excellent diuretic effect was obtained each time. The specific gravity of the urine varied between 1.012 and 1.018.

About 30 per cent of the phenolsulphonphthalein injected intramuscularly was excreted in two hours and ten minutes. Death occurred following a cerebral embolus.

The clinical diagnosis was arteriosclerosis with hypertension and heart disease; auricular fibrillation; paroxysmal tachycardia; congestive heart failure; infarct of heart, and cerebral embolus.

Pathologic Report.—A necropsy, performed twenty-seven hours after death, failed to verify the diagnosis of cerebral embolus. The kidneys were not enlarged. There were a few arteriosclerotic scars.

Microscopic preparations of the kidneys showed the architecture to be well preserved and the tissues well stained. The cells lining the convoluted tubules were low, the cytoplasm was granular, and the nuclei were vesicular.

The complete anatomic diagnosis was:

Primary: (*a*) Arteriosclerosis involving especially the coronary arteries; fibrosis of the myocardium; cardiac hypertrophy and dilatation; mural cardiac thrombi; organized pericarditis; ascites, and pulmonary infarcts.

Subsidiary: Apical pulmonary scars, and pleural adhesions.

Case 7.—*Clinical History.*—Mrs. E. R., a white woman, aged 70, had some dyspnea on exertion about three weeks before admission to the hospital. Following this she became orthopneic, and edema of the ankles developed. On admission to the hospital her temperature was 100.2 F., the pulse rate was 104 per minute, the respirations 42 and the blood pressure 114 systolic and 72 diastolic. On physical examination the heart was found to be slightly enlarged, and the liver was felt below the costal margin. After admission her course was steadily retrogressive. A large amount of fluid developed in the left side of the chest, and had to be removed by tapping on several occasions. The last of these, when the patient was practically dying, resulted in a pneumothorax. Death occurred a few hours later. Five-tenths cubic centimeters of merbaphen was given twenty-five days before death and 1 cc. nineteen days before death. Neither dose produced any diuretic effect. The specific gravity of the urine varied between 1.014 and 1.022. The phenolsulphonphthalein excretion was 20 per cent in two hours and ten minutes.

The clinical diagnosis was arteriosclerosis with hypertension and heart disease; congestive heart failure, and pneumothorax.

Pathologic Report.—Necropsy, performed one and one-half hours after death, showed that the kidneys were not enlarged. The architecture in the gross was not distorted.

Microscopic preparations of the kidneys showed the architecture to be well preserved and the tissue well stained. The cells lining the convoluted tubules were high, and the lumen was small. The cytoplasm was slightly granular, and the nuclei were vesicular.

The complete anatomic diagnosis was:

Primary: (*a*) Senility; passive congestion of the viscera; hydrothorax (bilateral); pulmonary atelectasis (bilateral); subcutaneous edema; thrombosis of uterine veins; emboli in pulmonary arterioles; fibrinous pleurisy (left), and acute hepatitis.

Subsidiary: Apical pulmonary scars (bilateral); carcinoma metastases to bronchial lymph nodes and lungs, and fibrous pleural adhesions (bilateral).

Case 8.—*Clinical History.*—E. G., a colored woman, aged 33, was brought to the hospital after dilatation and curettage of the uterus following an abortion. Four

months previously edema and dyspnea had appeared. Because of the poor condition of the patient, no further history was obtainable. On admission the temperature was 98 F., the pulse rate was 95 per minute, the respirations 30 and the blood pressure 205 systolic and 125 diastolic. Physical examination showed the patient to be dyspneic and orthopneic. The heart was slightly enlarged, and the extremities and abdomen were markedly edematous. The edge of the liver was felt at the level of the umbilicus. Her course in the hospital was steadily retrogressive. One cubic centimeter of merbaphen was given four days before death, with no diuretic effect. The specific gravity of the urine varied between 1.007 and 1.012. The blood nonprotein nitrogen was 89 mg. per hundred cubic centimeters.

The clinical diagnosis was arteriosclerosis with hypertension; heart and kidney disease; congestive heart failure, and syphilis (on the basis of a positive Wassermann test).

Pathologic Report.—Necropsy, performed two and one-half hours after death, showed small kidneys which were swollen and pale. The renal capsule was almost completely separated from the kidney by fluid. The cortical striations were not made out.

Microscopic preparations showed the typical picture of a chronic nephritis. The cells lining the convoluted tubules were well preserved, the cytoplasm being only slightly granular. The nuclei were vesicular.

The complete anatomic diagnosis was:

Primary: (*a*) Scars of the kidneys; hyalinized glomeruli; dilatation of the tubules; hypertrophy and atrophy of the renal epithelium; glomerular adhesions; cardiac hypertrophy and dilatation; fibrosis of the myocardium; congestion of the viscera; anasarca; focal lesions of the pancreas and liver, and acute diffuse nephritis.

Subsidiary: Thrombosis of the right ovarian vein; chronic cholecystitis and pericholangitis, and duodenal ulcers.

Case 9.—*Clinical History.*—F. R., a white man, aged 55, had had an anginal attack, lasting for fifteen minutes, three months before admission. Three weeks after this he had a similar attack, which was relieved only by morphine. Since then he had remained in bed. On admission to the hospital the temperature was 100 F., the pulse rate was 110 per minute, the respirations were 40 and the blood pressure 100 systolic and 90 diastolic. The patient was dyspneic and orthopneic. There were edema of the legs, ascites and an enlarged liver and heart. He did not improve with hospital care. Death occurred suddenly on the eighth day, apparently from occlusion of the coronary artery. He received 1 cc. of merbaphen the day before death. The specific gravity of the urine varied between 1.013 and 1.015.

The clinical diagnosis was arteriosclerosis with heart disease; infarct of heart, and congestive heart failure.

Pathologic Report.—Necropsy, performed five hours after death, showed that the kidneys were not enlarged and that the architecture was well preserved.

Microscopic preparations of the kidneys showed the architecture to be well preserved and the tissue to be well stained. The cells lining the convoluted tubules had extremely ragged edges, and many had sloughed off into the lumen. The cytoplasm was granular, and most of the nuclei were vesicular, although a few were deeply stained.

The complete anatomic diagnosis was:

Primary: (*a*) Arteriosclerosis, involving especially the coronary arteries; cardiac hypertrophy and dilatation; chronic passive congestion of the liver; mural thrombi in the ventricles; pulmonary infarcts (bilateral), and anasarca.

COMMENT

Merbaphen was given in three (1, 2 and 8) of the nine cases reported under conditions which are either known to be adverse to its use or might be so considered (fever, tuberculosis and chronic nephritis). In two of these cases (1 and 2) necrosis of the renal epithelium was found at necropsy. In case 8 no such damage could be found. Renal damage occurred in one (case 3) of the six remaining cases in which no known counterindications were present.

That it is impossible to obtain any correlation between the amount of merbaphen given and the renal damage which occurred is shown clearly in the accompanying table.

If case 1, in which the mercury was given by mistake after a rise in temperature, is ruled out, only two of eight cases remain in which

Tabulation of Clinical and Pathologic Observations

Case	No. of Doses	Total Amount Administered, Cc.	Time Between First Dose and Death	Time Between Last Dose and Death	Possible Counterindications	Diuresis	Damage to Renal Epithelium
1	3	4 to 5	25 days	3 days	Fever	+	++
2	2	3	32 days	30 days	Tuberculosis	+++	+
3	3	4.75	45 days	35 days	0	+	+
4	4	7.2	44 days	20 days	0	++	0
5	11	19	2½ years	49 days	0	+++	0
6	3	6	77 days	6 days	0	++	0
7	2	1.5	25 days	19 days	0	0	0
8	1	1	4 days	4 days	Nephritis	0	0
9	1	1	1 day	1 day	0	—	0

there was any anatomic damage to the kidney. It would appear, therefore, from this small series that the therapeutic use of merbaphen to produce diuresis is justifiable as a last resort.

SUMMARY

The clinical and necropsy reports of nine cases in which the patients received therapeutic intravenous injections of merbaphen are given. In one instance, the drug was given in the presence of a condition (fever) which is known to be adverse to its use. In two cases complications (tuberculosis and chronic nephritis) were present which may be considered as counterindications. One of these showed damage to the renal epithelium, and the other did not. Six of the nine cases showed no change, and the suggestion is made that the use of merbaphen is probably justifiable.

TRANSFUSION FROM A GROUP II (A) DONOR TO A GROUP III (B) RECIPIENT WITHOUT FATAL RESULT*

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This case is reported not only because the transfusion of a group II (A) blood to a group III (B) recipient has been almost universally accompanied by severe reaction or death, but also because serologic study of the present case has shown why the usual fatal result did not take place.

REPORT OF A CASE

Mrs. M. H., aged 35, colored, weighing only 85 pounds (38.6 Kg.), was hospitalized for a pelvic operation. Her chief complaint was lower abdominal pain of three months' duration, especially on the right side. Because of a secondary anemia of 55 per cent hemoglobin with 3,400,000 red blood cells, a preoperative transfusion was deemed advisable. The patient's blood, with the use of the usual microscopic method of grouping, was found to belong to group III (B). Her husband's blood was grouped by the same method and appeared to be that of a universal donor; that is, it belonged to the Jansky group I (O). Cross-matching of his cells versus the patient's serum appeared to be satisfactory.

The transfusion was started with the Scannell method. With the idea of preventing, if possible, the headache or dizziness that not infrequently accompanies transfusions, the blood was transferred to the patient considerably more slowly than usual. After from 40 to 60 cc. had been given, the patient began to cough occasionally and complained of some oppression in the chest and slight dizziness. The pulse remained of good quality, the rate being constantly around 90, and the patient looked well. The transfusion was continued very slowly until the patient complained of pain in the lumbar region of the back, when it was immediately stopped, this being at 200 cc. After from five to ten minutes these symptoms entirely disappeared, the patient appeared well, and the transfusion was continued slowly. When 400 cc. had been given, the transfusion was stopped entirely because of the recurrence of occasional coughing and slight oppression in the chest. These symptoms, however, did not persist, and there was no recurrence of the pain in the back. As the patient left the room to return to the ward, she complained of slight headache but nothing else; the pulse rate was still 90 and the pulse was of good quality; the blood pressure was 126 systolic and 85 diastolic, which was essentially the same as before the transfusion. Immediately after the transfusion the temperature was 99.2 F., the pulse rate 90 and the respirations 24; two hours later, the temperature was 101, the pulse rate 94 and the respirations 20. This was the only temperature reaction that occurred. At the end of six hours the headache had entirely disappeared. The following day

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the patient felt well; the blood showed 70 per cent hemoglobin with 3,850,000 red blood cells, and the urine was free from albumin, sugar and blood cells.

On the second day following the transfusion a right salpingo-oophorectomy and appendectomy was done, an adnexal mass from 2½ to 3 inches in diameter and an adherent, injected appendix being removed. The pathologic diagnosis was ovarian abscess, chronic salpingitis and chronic appendicitis, chiefly periappendicitis. The patient made an uneventful recovery, was allowed out of bed on the twelfth day, and was discharged on the eighteenth day after the operation.

Because of the immediate symptoms of reaction, it was thought advisable to check the grouping of the donor's blood. This was done by the same examiner, with the same method and with the same specimens of test serums. This time the blood of the donor was found to belong to a clearcut group II (A) instead of to the universal donor group, as was previously thought. This was checked by others, as was also the recipient's grouping. The mistake made in the first grouping and cross-matching of the donor's cells must have been due to the use of a very dilute suspension of cells, together with failure to stir or agitate sufficiently the mixtures of widely separated donor's cells and test serums. In the hope of finding an explanation for the remarkably mild character of the symptoms, the following further serologic study was carried out.

Specimens of blood were obtained from the recipient on the third and sixth days after the transfusion, and designated specimens 1 and 2, respectively. The results of the grouping with the usual test serums were different with the two specimens. When tested with group III serum (anti-A), specimen 1 showed a few large clumps in the midst of a great preponderance of unagglutinated corpuscles; the mixture with group II serum (anti-B) showed massive clumping of most of the cells with, however, a small proportion of cells lying singly and flat. The similar mixtures with specimen 2 showed no clumps in the test with group III (anti-A) serum and no flat-lying single cells in the test with group II (anti-B) serum. The tests with specimen 2 definitely identify the recipient as a group III individual, and the tests with specimen 1 demonstrate the presence of the donor's group II cells in small proportion in the recipient's blood. This method of detecting the presence of agglutinable transfused corpuscles has been used before by Jervell [1] in instances of transfusion of agglutinable corpuscles in an infant lacking both iso-agglutinins and agglutinable substances, and also in an adult under circumstances similar to the present case.

When the serum of specimen 1 was mixed on the slide with a 1:20 suspension of the donor's corpuscles at room temperature, there was a

1. Jervell, F.: Untersuchungen über die Lebensdauer der transfundierten roten Blutkörperche beim Menschen, Acta path. et microbiol. Scandinav. **1**:155, 1924.

rapid massive clumping of the cells. This result was surprising in view of the demonstrated presence of unagglutinated donor's corpuscles in the recipient's blood. The paradox was explained in the following experiment. The same constituents were used but were kept at body temperature both before and after the mixture was made. Examination of this mixture on a warmed slide showed no agglutination until the slide began to cool, when complete agglutination took place. Both this agglutination on the slide and that which occurred when the mixture in the test tube was allowed to cool were resolved on rewarming.

The foregoing experiments show that the patient's serum contained no specific agglutinins active at body temperature, but only a powerful property of agglutinating the donor's cells at room temperature or at a still lower temperature. In view of the well known fact that cold agglutinins are usually not group-specific, that is, they act on the blood corpuscles of all groups, it was necessary to determine whether the strong cold agglutination exhibited by the patient's serum was due to the presence of such nonspecific cold agglutinins, or whether, as is rarely the case, the agglutination was caused by the group-specific α which was capable of acting only at temperatures lower than 37 C.

This question was answered by testing the patient's serum against a number of blood specimens of the different groups: twenty-four of group I (O), twenty-four of group II (A), six of group III (B) and four of group IV (AB). In no instance was any clumping observed of group I or group III cells, and in every case the group II and group IV cells were strongly agglutinated at room temperature. None of the three blood specimens of group II tested at body temperature showed agglutination at this temperature. These tests identify the agglutinin as the group-specific α acting only in the cold. No similar instance has been found in the literature, although the unanalyzed case of Jervell may have been of the same nature.

Landsteiner and Levine [2] succeeded in demonstrating, along with the common iso-agglutinin, the existence of a fraction of the α iso-agglutinin which was incapable of clumping group II (A) cells at body temperature. In these experiments the cold α was accompanied by the usual α acting at body temperature. In a later paper,[3] a group A serum is described in which the β iso-agglutinin clumped B cells distinctly at room temperature but very weakly or not at all, depending on the blood cells, at body temperature.

2. Landsteiner, K., and Levine, Philip: On the Cold Agglutinins in Human Serum, J. Immunol. **12**:452, 1926.

3. Landsteiner, K., and Levine, Philip: On Isoagglutinin Reactions of Human Blood Other than Those Defining the Blood Groups, J. Immunol. **17**:23, 1929.

The serum of the patient (specimen 1) was examined for group-specific hemolysin for group II (A) corpuscles with and without the addition of the donor's serum. The protocol of this test is seen in the table which shows that group-specific hemolysin was absent.

The experiments explain the fortunate outcome of the transfusion. One may surmise that the mild symptoms observed during the transfusion were due to some cooling of the transfused blood sufficient to cause a mild temporary agglutination. In the accessible literature on transfusions I found only one instance of the transfusion of incompatible blood without death. This was reported by Jervell,[1] who transfused 600 cc. of group I (O) blood and then 600 cc. of group II (A) blood into a group III (B) recipient. He mentioned no immediate symptoms but noted urobilinuria and general icterus. No serologic study of this case was made.

Test for Hemolysin in Serums of Recipient and Donor

Five per cent suspension of donor's cells	cc.	0.2	0.2	0.2	0.2	0.2	0.2
Donor's serum	cc.	0.2	0.0	0.2	0.2	0.05	0.025
Patient's serum	cc.	0.0	0.2	0.2	0.1	0.05	0.025
Resulting hemolysis after 1 hour at 37 C.		0	0	0	0	0	0

The tests described were repeated in the presence of Philip Levine, Ella F. Grove and A. F. Coca, the results being confirmed.

SUMMARY

A full transfusion (400 cc. into a recipient weighing but 85 pounds) of group II (A) blood into a group III (B) recipient was accidentally performed, with only slight symptoms: coughing, oppression in the chest, slight dizziness and pain in the lumbar regions.

The incompatible transfused blood remained in the circulation of the recipient from three to five days.

The absence of serious consequences was shown to be due to the fact that all of the group-specific iso-agglutinin α in the recipient's serum was entirely incapable of clumping the group II (A) cells of the donor at body temperature, although it clumped these cells vigorously at room temperature.

PARATHYROID TUMOR AND CHANGES OF THE BONES*

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For some years, views regarding the origin and treatment of certain diseases of the skeleton have been expanded and improved. The following observations on a man aged 56, who had been bedridden for several years and whose condition had been diagnosed as osteomalacia, will serve in this connection.[1] The violent pain which the patient suffered defied all methods of treatment. He had given up all hope of recovery, and had come to the conclusion that he had an incurable disease. His only desire was that some chair or other apparatus could be constructed in which he would be able to rest without too much pain.

REPORT OF CASE

The patient was married and had a healthy wife and a healthy grown son. The somewhat complicated anamnesis showed that the patient had been well until 1922, when for the first time he noticed stiffness in his legs. In 1924 he fell to the pavement, hurting his knees, which became swollen and had to be aspirated. After having been in bed for two months he tried to get up, but could walk only with difficulty with the aid of two canes. He had continual pain in his legs, for which he was operated on in 1925. An operation for fractured patella was performed. After the operation, walking became even more difficult; the pain increased to such an extent that in 1927 another surgeon removed the iron wire with which the patella was held together in each knee. The tendon of the quadriceps muscle was sewn at the kneecap, but this afforded no relief. Since 1927 the patient had been unable to walk. By degrees his body became so tender to touch that it was an almost unbearable torture for him to have his bed made, and nursing was thus almost impossible. A spontaneous fracture of the right thigh-bone which occurred in January, 1929, when the patient was being carefully shifted in bed, further complicated his condition.

It must be emphasized that in 1925 a swelling developed on the top of the right foot; this gradually increased in size. The patient, who formerly had been extraordinarily strong and able to lift and push heavy weights, now grew weaker and weaker as the severe pains impaired his appetite and his sleep. He became emaciated and nervous, although he did not suffer from palpitation or perspiration. He had no cough, and his bowels moved regularly.

Even without roentgen examination, the diagnosis of osteomalacia or an analogous disease had to be considered, as in a few years the man had diminished in size; his back had become curved like a bow, and his thorax compressed. On the other hand, the lower thoracic aperture was dilated and nearly reached the

* Submitted for publication, Jan. 6, 1930.

1. Snapper: Verhandl. d. deutsch. Gesellsch. f. inn. Med. **41**:183, 1929; Nederl. tijdschr. v. geneesk. **2**:47, 1929.

upper border of the pelvis. The thighs were bent, and the feet lay in outward rotation. The thorax and the legs especially were extremely tender on pressure. On account of this enforced restriction in movement, the general examination was especially difficult. The heart and lungs, peripheral and central nervous system appeared normal, so far as they could be examined. Nothing abnormal was found in the abdominal organs. There was, however, extensive pitting edema of the legs, and a great number of leukocytes were present in the urine. The blood pressure was normal; the blood picture showed no abnormal cells; the Wassermann test was negative.

In September, 1928, a roentgen examination had shown extensive osteomalacic changes of the bones (Dr. Heilbron). The spine, pelvis and thighs were totally decalcified. The pelvis, for example, had become quite transparent; the natural

Fig. 1.—Pelvis before operation.

compression had brought about the typical form of wedge-shaped osteomalacic pelvis. The decalcification was less clearly pronounced in the arms, lower part of the legs, hands and feet. A nonconsolidated fracture was found on the right thigh bone. Although in a great part of the skeleton a marked decalcification existed, which in many respects reminded one of osteomalacia, the definite diagnosis of true osteomalacia seemed doubtful.

Osteomalacia, strictly speaking, is a disease which is characterized by decalcification of the bones, and it occurs especially during pregnancy. In children and in male adults extensive decalcification can also be observed. It is, however, inadvisable to consider these as cases of true osteomalacia, because most of these patients are suffering from osteomalacic end-stages of other diseases of the bones. In the present

case, therefore, in which the patient was a man aged 56, pseudo-osteomalacia seems more probable. From time to time similar decalcifications of large parts of the skeleton are met with in cases of dissemination of malignant metastases in the bones. This possibility received serious consideration in the present case, but a primary tumor was not found.

Fig. 2.—Femur before operation.

Later, during a more extensive examination of the patient, I stumbled on the fact that the blood serum contained an abnormally high amount of calcium. While, as a rule, the serum calcium of man is stable and fluctuates only between 9 and 12 mg. per hundred cubic centimeters of serum, unusually high calcium contents were found on four different occasions in the serum of the patient, viz., 19, 21.4, 22.8 and once even

23.6 mg. per hundred cubic centimeters. This unduly high serum calcium content, hypercalcemia, proved to be the principal landmark which led to the correct diagnosis. A more thorough discussion of the general significance of such a hypercalcemia is therefore indicated.

Increase in the serum calcium is rarely met with, while, on the other hand, decreased serum calcium or hypocalcemia, belongs to the characteristic features of tetany, which is caused by damage to the parathyroids.

If, therefore, hypocalcemia is a symptom of impaired function of the parathyroids, hypercalcemia, as observed in this patient with so-called osteomalacia, should point to hyperfunction of the parathyroid.

During the last decade it has become obvious that a connection exists between certain diseases of the skeleton and changes in the morphology and function of the parathyroids; the hypercalcemia in this case, therefore, suggested that possibly a hyperfunction of the parathyroids might be the cause of the disease.

In 1904, Askanazy described a tumor of one parathyroid found in a case of osteitis deformans, after Erdheim had previously discovered an enlargement of these glands in patients suffering from osteomalacia. Since these reports appeared, the number of cases in which a tumor of a parathyroid was found in patients suffering from osteitis fibrosa generalisata has steadily grown. In 1925, Hoffheinz collected from the literature forty-five cases of parathyroid tumor. Of these forty-five patients, twenty-seven suffered from various diseases of the bones, viz., seventeen from osteitis fibrosa generalisata, eight from osteomalacia and two from rachitis. From these observations it has become evident that in all cases of osteitis fibrosa generalisata or Recklinghausen's disease, the possibility of a parathyroid tumor should be borne in mind.

Osteitis fibrosa generalisata is characterized by a fibrous degeneration of the bone, accompanied by the formation of cysts. So-called Recklinghausen's disease, therefore, is usually recognized by multiple foci of cystic degeneration of the bones. These cysts may be filled with brown, semiliquid material, and pseudosarcomatous giant cell growth often occurs in them, especially if they are situated in the distal ends of the long shafts. Clinical observation has proved, however, that these so-called giant cell sarcomas, which are so frequently observed with osteitis fibrosa generalisata, are always benign; biologically, they are not to be looked on as malignant tumors. Severe pain originating from the bone-marrow is seldom lacking, and it complicates the clinical picture of Recklinghausen's disease. Spontaneous fractures are also not infrequent. The anatomic examination proves that this disease is characterized by both too rapid destruction and too rapid construction of bone tissue. The rapid construction, however, gives rise to inferior osteoid bone tissue which does not calcify. In the long run, the normal

bone is replaced by uncalcified osteoid tissue; finally decalcification is so extensive that the clinical symptoms may be identical with those of osteomalacia. True puerperal osteomalacia, however, may be distinguished anatomically from this pseudo-osteomalacic end-stage of osteitis fibrosa generalisata.[2]

It must be mentioned that there is much histologic resemblance between Recklinghausen's disease and the well known osteitis deformans Paget with its increase in the size of the scalp and in the thickening of the compacta of the long shaft bones. However, in agreement with the fact that the clinical picture of Recklinghausen's disease and that of Paget's disease are quite different, it must be noted that in Recklinghausen's disease an adenoma of a parathyroid is frequently observed, whereas in Paget's disease this tumor is hardly ever found.

Until a short time ago there was a difference of opinion with regard to the question of whether the tumor of the parathyroid was the cause or the result of the changes in the bones. Erdheim[3] defended the opinion that primarily disease of the bones was present. The large quantities of calcium that are released by the degeneration of the skeleton make such great demands on the organs of the calcium metabolism (in this case, the parathyroids) that a compensatory hypertrophy of these glands results. This explanation, however, can hardly be brought forward in face of the fact that in osteitis fibrosa, as a rule, only one of the parathyroids is hypertrophied, while the others remain normal. Yet there are important arguments in favor of Erdheim's conception. Klemperer's observation, in particular, in which he noted a parathyroid tumor in a case of generalized carcinosis of the bone after mammary carcinoma, must be mentioned. In this case the decalcification through carcinosis seems to have been primary, whereas the tumor of the parathyroid was secondary. There are arguments, however, which point to the fact that Klemperer's[4] observation probably is an exception to the rule, and that in most cases of osteitis fibrosa generalisata the tumor of the parathyroid must be considered as the cause of the disease of the bone. Günther[5] also defended this opinion on the basis of anatomic observations.

This view found important support in a further analysis of the action of parathyroid extract-Collip. Injection of this substance causes increase of the calcium content of the serum in man and animals and

2. These facts are based on detailed reports in the discussion of the Deutsche Pathologische Gesellschaft in Freiburg, 1926.

3. Erdheim: Beitr. z. path. Anat. u. z. allg. Path. **33**:214, 1903; Frankfurt. Ztschr. f. Path. **7**:175, 1911.

4. Klemperer: Surg. Gynec. Obst. **36**: 11, 1923.

5. Günther: Frankfurt. Ztschr. f. Path. **28**:319, 1922.

also an increase of calcium output in the urine. Collip proved that this hypercalcemia is caused by the fact that parathyroid extract increases calcium absorption from the skeleton. If the injections are continued long enough, the skeleton loses so much calcium that spontaneous fractures may occur. One must consider, further, that not only Recklinghausen's disease but the continuous supply of parathyroid extract is accompanied by hypercalcemia, increased calcium secretion in the urine and spontaneous fractures. It is therefore probable that by increased production of parathyroid hormone the parathyroid tumor found in Recklinghausen's disease causes the greatest number of the symptoms of this illness.

Mandl, a surgeon from Vienna, has accepted this conception. Until a few years ago, Viennese scientists, especially, took interest in the problem of the occurrence of parathyroid tumors in cases of osteitis fibrosa generalisata and analogous diseases. On account of Erdheim's investigations, this question was repeatedly a subject of discussion at scientific meetings in Vienna. In 1915, during one of these discussions, Schlagenhaufer raised the question as to whether one could not try to extirpate the tumor of the parathyroid in cases of osteitis fibrosa generalisata. Maresch supported this suggestion, but it was ten years before Mandl actually performed this operation (1925).[6] For several years, on account of a generalized osteitis fibrosa, Mandl's patient, aged 38, had been able to walk only with the greatest difficulty and with the assistance of crutches; during the last months prior to the operation, he was bedridden on account of terrible pain. After the operation, which consisted of the removal of the parathyroid tumor, the patient gradually grew better. Although roentgenograms showed little improvement in the calcium content of the skeleton, the patient recovered sufficiently to work again like a man in an ordinary state of health.

Two years later Gold,[7] also in Vienna, successfully operated on a second patient. In 1929, analogous cases were observed by Du Bois,[7a] Barr[8] and Wilder.[9] In these American cases, also, clinical recovery from Recklinghausen's disease was obtained by operations on the parathyroid.[10] In the American cases a roentgenologic improvement

6. Mandl: Arch. f. klin. Chir. **143**:1, 1926.

7. Gold: Mitt. a. d. Grenzgeb. d. Med. u. Chir. **41**:63, 1928; Klin. Wchnschr., 1929, p. 2247.

7a. Hannon, R. R.; Shorr, E.; McClellan, W. S., and Du Bois, E. F.: J. Clin. Investigation **8**:215, 1930.

8. Barr, D. P.; Bulger, H. A., and Dixon, H. H.: Hyperparathyroidism, J. A. M. A. **92**:951 (March 23) 1929.

9. Wilder: Endocrinology **13**:231, 1929.

10. Boyd, J. D.; Milgram, J. E., and Stearns, G.: Clinical Hyperparathyroidism, J. A. M. A. **93**:684 (Aug. 31) 1929. Hunter, D.: Proc. Roy. Soc. Med. **23**:27, 1929.

in the calcium content of the skeleton was always observed after operation. Taking all these observations into account, it is highly probable that the parathyroid tumor is the cause of the osteitis fibrosa generalisata.

In the present case it was essential, therefore, to consider the possibility of a tumor of a parathyroid. It had to be borne in mind, however, that only osteomalacic changes were found, while nothing was known of osteitis fibrosa generalisata. Although parathyroid tumors may

Fig. 3.—Osteitis fibrosa cystica of the second metatarsal (taken in 1925 by Dr. Morlet, Antwerp).

be found at times in osteomalacia, these tumors are observed as a general rule in cases of osteitis fibrosa generalisata. Fortunately the patient had had roentgenograms taken in Antwerp in 1925 for the swelling on the right foot, as previously described. Dr. Morlet of Antwerp put them at my disposal. It appeared from these that a real osteitis fibrosa cystica of the second metatarsal of the right foot existed in 1925; they also showed an accumulation of cysts in the fossa glenoidalis of the scapula. The disease originated, therefore, as a real osteitis fibrosa cystica generalisata. The deduction could therefore be made that the condition was

the pseudo-osteomalacic final stage of Recklinghausen's disease. By this observation, moreover, the possibility of a tumor of the parathyroid was still further increased. Accurate palpation of the thyroid region showed that there was a small tumor, about the size of a plum stone, in the left lobe of the thyroid gland; this small growth moved up and down during the act of swallowing. A small adenoma of the thyroid itself could not be excluded, but on the basis of the facts mentioned it seemed clear that one could rather look on this small lump as a tumor of a parathyroid. At that time, in February, 1929, only the cases of Mandl and Gold were known. The remarkable results obtained by these surgeons led to the extirpation of the parathyroid tumor of this patient.

EXTIRPATION OF PARATHYROID TUMOR

On Feb. 28, 1929, Professor Lanz exposed the left side of the thyroid. In lifting the left lobe, a normal parathyroid was to be seen at the upper pole of the

Fig. 4.—Parathyroid tumor, cut open.

left lobe of the thyroid. At the lower pole a slight, somewhat yellowish protrusion could be seen on the spot where the lower parathyroid was to be expected, viz., where the thyroid artery enters the thyroid gland and the recurrent nerve comes into intimate contact with the thyroid capsule. From this situation a tumor, 25 by 14 mm. in size, was enucleated after incision of the capsule of the thyroid. As it is known that in Recklinghausen's disease the tumors of the parathyroid are nearly always solitary, the right lobe of the thyroid was not inspected.

On section, the tumor was found to consist chiefly of a yellowish compact parenchyma, while one-third was taken up by a cyst. Microscopic examination by Professor de Vries revealed that the growth was an adenoma of a parathyroid.

The further clinical course in this case showed that this adenoma of the parathyroid was the cause of the hypercalcemia.

Two symptoms which seemed favorable were noticed on the day after the operation, while two other symptoms gave reason for uneasiness. The first of the favorable symptoms was that the tenderness of the skeleton seemed to be less, the patient's legs could be moved a trifle more easily, and the thorax was also less painful when pressed. In the second place, the calcium content of the serum had gone down from 21.6 to 14.9 mg. per hundred cubic centimeters twenty-four hours after the operation; the decalcification of

the skeleton had been influenced favorably. The two less favorable symptoms were: (1) an obvious exophthalmos, probably caused by manipulation of the thyroid, an excessive amount of thyroid hormone having been pressed into the circulation during the operation; this symptom could be observed for only two days, after which it gradually disappeared; (2) the secretion of urine appeared to be disturbed. While at least 2 liters of urine was secreted daily before the operation, only 800 cc. was secreted on the day of the operation, and on the day after the operation, in spite of ample liquid supply, only 150 cc. This oliguria was especially unpleasant in this case, because the patient had severe pyuria and damaged kidneys. Even before the operation, with rest in bed and limited nourishment the nonprotein nitrogen of his blood was 31 mg. per hundred cubic centimeters.

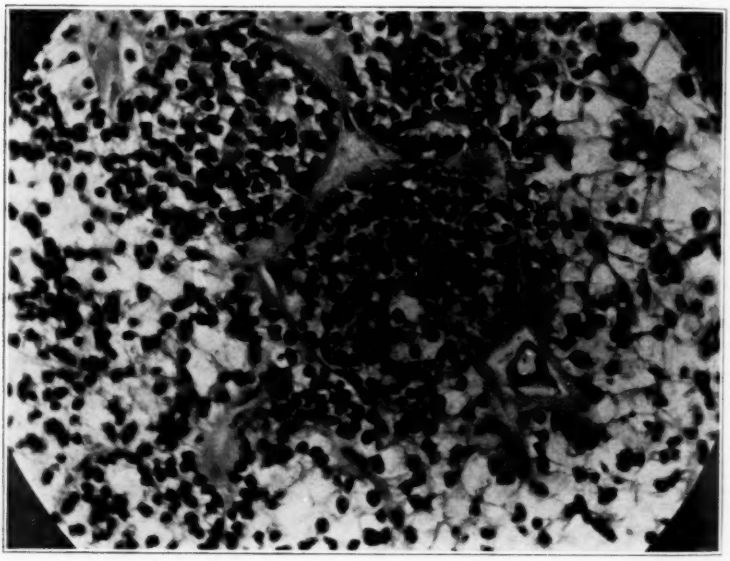

Fig. 5.—Microscopic section of tumor.

During the oliguria the nonprotein nitrogen rose as high as 55 mg. per hundred cubic centimeters. Fortunately, 750 cc. of urine was secreted on the second day after the operation and 1,600 cc. on the third day; after this the diuresis increased to 2 liters daily and more.

The tenderness of the bones diminished day by day during the first three weeks after the operation, but the mental condition of the patient gave reason for anxiety. He became irritable and was often discontented with his surroundings; sometimes he took the hand of someone standing near and began to weep; there were hours in which he sat staring silently into space. An obvious tremor developed, which lasted sometimes for hours, followed by a short interval, when it began again. These symptoms developed after the operation. The patient suffered from insomnia, as well, but his sleep had been affected before the operation. This strange mental

disturbance, which made nursing and feeding difficult, was explained by the rapid decrease of the calcium content of the serum. The amount of calcium per hundred cubic centimeters of serum was:

Before operation	21.4 mg.
24 hours after operation	14.9 mg.
5 days after operation	8.5 mg.
17 days after operation	7.2 mg.

These mental symptoms were apparently signs of impending tetany; when the calcium content of the serum had gone down to 7.2 mg. per hundred cubic centimeters, Chvostek's symptom, i. e., rapid contraction of the corner of the mouth and nostrils through percussing the facial nerve in front of the concha, could be observed for the first time. Five days later, that is, three weeks after the operation, the calcium content had gone down to 6.60 mg. per hundred cubic centimeters. The patient now lost all count of time and place; he screamed and yelled at night; he hardly recognized his surroundings, and if his osteomalacic limbs had permitted it, he would have tried to jump out of bed. Clearly, this amentia called for heroic measures. Although the patient had taken 4 Gm. of calcium lactate daily by mouth, since the operation, this apparently was not sufficient to keep the calcium content of the serum on a normal level. He now received injections of parathyroid extract-Collip subcutaneouly and of a calcium compound intravenously.[11] The injection of 30 units of parathyroid extract daily and 10 cc. of the calcium compound twice daily raised the calcium content of the serum to 8 mg. per hundred cubic centimeters within three days. The patient became quieter, the amentia disappeared, and an agreeable euphoria gladdened both the patient and persons around him. This treatment proved necessary during a period of four weeks. The dosage was 30 units of parathyroid extract daily for the first two days, followed by 20 units for four days; for the remainder of the four weeks, the patient was given, on alternate days, 20 units and 10 units, respectively. The calcium was injected intravenously twice daily at the beginning of the treatment, and afterward only once a day. The calcium content of the serum went up to 9.04 mg. per hundred cubic centimeters after four weeks. The injections of parathyroid extract and calcium were stopped, and the calcium content of the serum remained on the normal level with daily doses of 4 Gm. of calcium lactate administered by mouth. The patient improved quickly during this period. The tenderness of the thorax was the first symptom to disappear, and the legs could now be moved more easily. The patient, further, was able to make active movements with his feet and toes. Six weeks after the operation, the patient was moved for the first time to a couch. At first, movement was accompanied by sighs and groans, but after some weeks, the patient for the first time tried walking between two nurses. Rapid improvement was made in this respect, so that at the beginning of August the patient was able to walk a short distance with no other assistance than two canes. Early in October he could walk for some minutes with one cane only. The tenderness of the skeleton had entirely disappeared. The patient's appetite was good; sleep and other vegetative functions were undisturbed. The edema of the legs had diminished considerably and had made room for hard induration, so that the patient was again able to wear his shoes. In short, as a result of the operation,

11. Helfenberg describes this calcium compound as one which does not cause irritation even if a drop gets outside of the vein.

an entire change had been brought about and clinical recovery has been obtained. The patient, it will be readily understood, remained a small, bent man with crooked legs, but the unfortunate invalid, chained to his bed in agony had been changed into a cheerful man, who had no more pain and who recorded with delight his daily improvement in walking.

COMMENT

In studying this peculiar case various details remain to be discussed. The subjective improvement was accompanied by an objective improvement in the calcium metabolism. Before the operation the calcium was dissolved from the bones through excessive secretion of parathyroid hormone by the tumor. This calcium circulated in the blood

TABLE 1.—*Determinations of Calcium in the Serum*

Before Operation		After Operation		
Date	Mg. per 100 Cc.	Date	Mg. per 100 Cc.	
2/13/20.....	23.6	3/ 1/20.....	14.9	Nonprotein nitrogen, 55 mg. per 100 cc.
2/14/20.....	22.8	3/ 5/20.....	8.50	Nonprotein nitrogen, 51 mg. per 100 cc.
2/22/20.....	19.0	3/17/20.....	7.20	
2/27/20.....	21.4	3/22/20.....	6.60	
2/27/20, nonprotein nitrogen	31	3/25/20.....	8.00	Calcium intravenously and parathyroid extract subcutaneously
		4/ 4/20.....	7.67	Calcium intravenously and parathyroid extract subcutaneously
		4/ 9/20.....	7.78	Calcium intravenously and parathyroid extract subcutaneously
		4/20/20.....	9.86	Calcium intravenously and parathyroid extract subcutaneously
		4/23/20.....	9.04	Calcium intravenously and parathyroid extract subcutaneously
		5/ 1/20.....	8.83	
		5/15/20.....	8.15	
		5/28/20.....	9.04	
		6/ 4/20.....	9.66	
		7/12/20.....	10.56	Nonprotein nitrogen, 64 mg. per 100 cc.
		9/27/20.....	11.20	Nonprotein nitrogen, 51 mg. per 100 cc.

and caused the high calcium content of the serum and also of the urine, in which, as will be seen presently, abnormally large quantities of calcium were secreted. After the operation, the calcium content of the serum immediately went down. Table 1 shows how, as has already been mentioned, the calcium content of the serum fell within three weeks from 20 to 23 mg. to below 7 mg. per hundred cubic centimeters. Subsequently, by injecting calcium intravenously, and parathyroid extract subcutaneously, over a period of four weeks, the serum calcium was brought up to 9 mg. per hundred cubic centimeters. After this period, even without special treatment, the calcium content of the serum remained on a normal level. Following the operation the calcium secretion in the urine was completely changed (table 3). Before the operation, the patient was given a diet on which control experiments had showed normal persons secreted about from 30 to 100 mg. of calcium daily in the urine. This patient, however, when on this diet before his operation, secreted more than 400 mg. of calcium daily. After the

operation only from 25 to 27 mg. of calcium was found in the urine every twenty-four hours, if the patient observed the aforementioned test diet (table 2). A fortnight after the operation the calcium secretion in the urine had become normal, which is clearly a proof that extirpation of adenoma of the parathyroid removed at the same time the cause of

TABLE 2.—*Other Observations on the Serum and the Blood Before Operation*

Substance	Mg. per 100 Cc.	
Chloride	380	
Calcium	21.4	
Phosphorus pentoxide	2.1	
Cholesterol	200	
Lactic acid	30	
Uric acid	5.8	
Total albumin	6940	
Albumin : globulin	7.02 : 2.98	
Nonprotein nitrogen	31	
Amino nitrogen	6.57	
Alkali reserve	51 per cent by volume	
Fasting blood sugar	89	
50 Gm. of dextrose by mouth:		
After ½ hour	125	
After 1 hour	157	No glycosuria
After 1½ hours	244	
After 2 hours	150	
Bleeding time	2½ minutes	
Coagulation time:		
Beginning	7½ minutes	
Total	8½ minutes	

TABLE 3.—*Results of Urinalysis with the Patient on a Calcium-Test Diet*

Date, 1929	Quantity, Cc.	Chlorine, Gm.	Calcium, Mg.	Phosphate, Mg.
Before operation				
2/24	2,400	2.88	411	883
2/25	2,300	3.2	393	925
2/26	2,500	4.04	425	874
2/27	2,400	2.66	432	971
2/28	2,200	3.2	322	880
After operation (2/28)				
3/14	2,300	5.1	2.2	402
3/15	1,900	4.1	25.0	346
3/16	2,000	4.2	37.0	375
4/22	1,380	3.76	23.5	420
4/23	1,500	3.45	28.8	564
4/24	1,500	4.1	39.1	538
5/10	1,100	....	30.7	
5/11	1,680	....	41.5	
5/28	1,700	....	37.6	
5/29	1,580	....	48.0	

the abnormal calcium metabolism, i. e., the cause of the dissolving of the calcium from the bones. It appears, further, that the pain was closely connected with this dissolving of the calcium from the skeleton. As soon as the calcium metabolism became normal, the pain in the bones disappeared. The other writers on the subject also observed, without exception, that their patients secreted abnormally great amounts of calcium in the urine before the operation, and that after the operation the calcium secretion went down to the normal rate of from 30 to 50 mg. per twenty-four hours. It may be asked whether, together with

the improvement in the calcium metabolism, a calcification of the osteomalacic bones cannot also be observed. Mandl first reported that he had observed this in his case. Later, however, he had to admit that in spite of the really remarkable clinical improvement in his patient, roentgenologically only slight changes were noted on the skeleton. Gold

Fig. 6.—Femur, six months after operation.

also came to this conclusion. The American authors, however, observed roentgenologic improvement in all their cases.

When examined a considerable time after the operation, the pelvis of the patient showed little change. In October, 1929, it was still a real osteomalacic, transparent, wedge-shaped pelvis. An important change could be observed, however, in the right femur. The fracture had healed with callus formation, and all the bone structures of the femur

had become more compact. In February, 1930, the pelvis also showed extensive recalcification.

The extraordinary and striking arteriosclerosis of the patient remains to be mentioned. On the roentgenogram one can see the whole arteria femoralis appearing like a calcified snake in the muscle mass. On some roentgenograms even the ramification of the arteria femoralis profunda is to be seen. This gives one the impression that the vascular wall had greedily absorbed the excessive quantity of calcium in the serum. Clinical improvement did not change this marked arteriosclerosis; the calcium was apparently firmly embedded in the vascular wall.

The question as to why the calcium content of the serum of this patient was so much higher than the amounts which the other authors found in their cases of parathyroid tumor with osteitis fibrosa generalisata calls for special discussion. While in this patient the calcium content in the serum fluctuated between 19 and 23 mg. per hundred cubic centimeters, Gold found 13 mg. per hundred cubic centimeters, Du Bois and Aub 14.5 and 16 mg., Barr 16 mg. and Wilder 11.2 mg. In order to explain the much greater hypercalcemia in my patient, one has to bear in mind in the first place that he had a much more advanced case of osteomalacia than did the other patients, in whom more symptoms of real osteitis fibrosa generalisata were found. For this reason, probably, the decalcification was more pronounced in this patient, and thereby, more calcium was dissolved from the bones. In the second place, one must also call to mind that the patient had damaged kidneys. Many patients suffering from osteitis fibrosa generalisata produce calcium stones in the kidneys (e. g., Barr's patient) through large amounts of calcium secreted with the urine. Neither roentgenologic nor clinical symptoms of kidney stones were to be found in this patient. His pyuria, however, was a sign of pyelonephritis, which may or may not have been calculous, but through which the excretory function of the kidneys suffered. This was proved by the nonprotein nitrogen of the blood. The nonprotein nitrogen of the blood of 31 mg. per hundred cubic centimeters before the operation was too high, when one considers that this patient was then closely confined to his bed and was on a much restricted diet. During the anxious days of the oliguria directly after the operation, the nonprotein nitrogen of the blood rose to 55 mg. per hundred cubic centimeters. When the diuresis started again, the nonprotein nitrogen of the blood did not go down, but remained on this level. To make quite certain, the nonprotein nitrogen of the blood was reexamined in August, 1929. At that time the patient was exceedingly well; he had a normal blood pressure and a tremendous appetite and did not complain of headache or nausea. Notwithstanding this general improvement in health, the urea content of the blood was then 1 Gm. per liter, and the nonprotein nitrogen of the blood 64 mg. per

hundred cubic centimeters. In October, 1929, the blood pressure was 135 systolic and 90 diastolic, and the nonprotein nitrogen 51 mg. per hundred cubic centimeters. There was, therefore, a decreased permeability of the kidneys; this is probably one of the reasons why the calcium content of the serum rose to an abnormal height.

It must be emphasized that the impaired kidney function alone cannot be the cause of the hypercalcemia. This cannot be the case because (1) after the operation, in spite of the impaired function of the kidneys, the calcium content of the serum went down immediately, and (2) in real uremia the figures for the serum calcium never reach this level. Therefore, the damaged kidney function can be only one of the causes of the abnormally high rate of hypercalcemia.

When one considers the fact that the calcium of the bone consists chiefly of calcium phosphate, it is obvious that a disturbance of the calcium metabolism must often involve disturbance of the phosphate metabolism. Collip has pointed out that if the calcium content of the serum increases under the influence of injections of parathyroid extract, the phosphate content of the serum decreases. In Wilder's case, the phosphate content of the blood was only 1.4 mg. per hundred cubic centimeters; in Barr's case, 1.4 mg.; in Dubois and Aub's case, 3.3 mg. and in the present case, only 2.1 mg., whereas the normal phosphate content is about 3 mg. While in Wilder's patient the phosphates of the blood remained low after the operation, viz., about 1.5 mg. per hundred cubic centimeters, in my patient it went up to 3.3 mg. five months after the operation.

Before the operation the phosphate secretion of the patient, on the same diet as that used in the examination for calcium, varied between 880 and 717 mg. daily. After the operation only from 364 to 560 mg. of phosphate was secreted daily in the urine. In Wilder's case the phosphate secretion also went down from 650 mg. daily before the operation to 300 mg. daily after the operation.

In discussing the present case, it has become evident that for the diagnosis of a parathyroid tumor with an osteitis fibrosa generalisata the finding of hypercalcemia is of the greatest importance. One has to point out, however, that in Wilder's case the calcium content of the serum hardly increased. Once a rate of 10.7 mg. of calcium per hundred cubic centimeters was found in the serum of the patient, and the highest figures found before the operation were between 11.8 and 13.7 mg. per hundred cubic centimeters.

On account of the fact that a parathyroid tumor existed in this case while the calcium content of the blood serum was nearly normal, in the future the calcium secretion in the urine, as well as the calcium content of the blood serum, will be analyzed in doubtful cases in order to avoid the exclusion of a parathyroid tumor, for the sole reason that the calcium content of the serum is about normal.

Furthermore, stress must be laid on the fact that although the calcium content of the serum and that of the urine form important elements, clinical experience still remains the chief factor in the ultimate diagnosis. As a matter of fact, in other diseases a high calcium content of the blood may be present, for example, in malignant myelomas (Barr). At the Mayo clinic this has been observed in polyglobulia, and it has also once been described in a case of rachitis tarda.

On the other hand, highly increased calcium secretion in the urine, with a normal calcium content of the blood serum, is almost constantly found by Aub in cases of exophthalmic goiter; a similar condition to that found in Wilder's case then arises. In short, diagnostic errors may easily occur, and however important the data of the chemical analysis of blood and urine may appear, in the diagnosis of a parathyroid tumor clinical skill and experience must play an even more important part.

Sometimes the tumor of the parathyroid may be discovered through palpation before operation; sometimes, however, a parathyroid tumor may be present which is not revealed through palpation (Mandl, Gold). Wilder's patient had a malignant adenoma of the parathyroid (Wellbrock[12]). In Du Bois' case no tumor was found even at operation. Clinical recovery was obtained by the removal of two normal-looking parathyroids.

The oliguria which was observed in the present case after extirpation of the tumor deserves special mention. Gold also mentioned oliguria several days after the operation. In this connection McCann's experiments must be recalled;[13] by injections of parathyroid extract he was able to obtain the same diuretic effect, which may be observed also after the administration of thyroid extract to patients with edematous kidneys. The hormone of the parathyroid apparently has an influence on the physicochemical properties of the tissues. In this way it may be understood how after removal of the principal source of the parathyroid extract, i. e., the adenoma, in the case reported, the output of urine diminished. The patient evidently was accustomed to excessive quantities of parathyroid hormone. It took several days before the organism became used to the much smaller quantity of parathyroid hormone secreted by the remaining parathyroids.

Finally, there is the most important problem, the fact that severe tetany can arise after removal of a parathyroid tumor. Beck has described how through tetany he lost a patient after he had removed a tumor of a parathyroid gland.[14] In Barr's patient also serious tetany

12. Wellbrock: Endocrinology **13**:285, 1929.

13. McCann, W. S.: Diuretic Action of Parathyroid Extract-Collip in Certain Edematous Patients, J. A. M. A. **90**:249 (Jan.) 1928. Hueper, W. C.: The Diuretic Action of the Parathyroid Extract, Arch. Int. Med. **44**:374 (Sept.) 1929.

14. Beck: Arch. f. klin. Chir. **152**:123, 1928.

developed two days after the operation, and many months afterward she still needed calcium and parathyroid extract. The development of tetany after the operation can be explained in various ways. The possibility of an injury to the other parathyroids during the operation is obvious. In the present case, however, the right side of the thyroid was not exposed. The parathyroids situated at the right side could not be injured, therefore, during the operation. The possibility also exists that this patient had no parathyroids on the right side, and that immediately after the operation the only remaining left parathyroid gland was not able to regulate the calcium metabolism adequately. Meanwhile, if one does not wish to use the unproved hypothesis that the patient had no parathyroids on the right side, another possibility may be considered. After the operation the decalcified skeleton may absorb calcium from the blood with so much avidity that through this rapid absorption the calcium content of the serum descends below the critical level, and symptoms of tetany appear; even three normal parathyroids would then be unable to regulate this absorption through the skeleton. Only a mechanism of this order could explain the symptoms of tetany if the patient had normal parathyroids on the right side. In Barr's case there was a strong argument in favor of this conception; up to 100 units of parathyroid extract were injected daily into his patient when symptoms of tetany developed after the operation. In spite of these injections, her condition and the symptoms of tetany grew worse. At that time this patient had sufficient parathyroid hormone (injected), but nevertheless suffered from tetany. Only when calcium chloride was injected intravenously at the same time as the parathyroid extract did improvement occur. Before the treatment by injections of calcium Barr's patient was given 4 Gm. of calcium by mouth; this oral administration had no influence on the tetany. This observation is also in favor of the hypothesis that even with sufficient parathyroid function, in other words, with sufficient parathyroid extract, tetany may arise in these patients through excessive absorption of calcium from the serum.

Parathyroid extract was given to my patient only when his condition became precarious. Perhaps tetany could have been avoided altogether if parathyroid extract had been given earlier. It is clear, however, that increase of the calcium content of the serum under the influence of parathyroid extract is brought about by resorption of calcium from the bones, an occurrence that I wished to avoid. In future cases I shall without doubt inject calcium intravenously directly after the operation. It seems evident that after the long negative calcium balance, there exists such a calcium hunger of the organism that in these conditions administration of calcium by mouth is not sufficient.

Because there is danger of tetany after an operation for a parathyroid tumor, it may be asked whether recovery can occur without

operation. Regnier [15] made an important observation. He obtained considerable improvement in a patient with Recklinghausen's disease by giving large doses of irradiated ergosterol. Gold remarked in the discussion that he doubted the diagnosis of osteitis fibrosa generalisata in Regnier's case, but Kienböck declared subsequently that there was no doubt that Regnier's diagnosis was correct. Blumgart [16] of Boston noticed improvement in a case of osteomalacia after treatment with irradiated ergosterol, but he stated that his patient suffered from rachitis tarda. On the other hand, other clinicians were not successful with this treatment. Wilder's patient lived from the end of May, 1927, to May 16, 1928, on a diet which contained a great deal of vitamin D; on this diet, combined with treatment by alpine light, the patient grew stronger and heavier, and the calcium and phosphate balance became positive. The calcium content of the serum, however, did not change, and the pain remained the same, so that an operation finally had to be performed. The patient described here also took irradiated ergosterol for some time before his operation, but without success. It is clear that even if a trial of irradiated ergosterol is justified, operation for the tumor of the parathyroid may still be necessary in many cases.

In conclusion, it may be stated with confidence that the lives of patients suffering from osteitis fibrosa generalisata can be saved by removal of the adenoma of the parathyroid. This operation will protect them from severe illness of many years' duration, an illness which leads, as a rule, to a cachectic end-stage and ultimate death in violent pain. Finally, it must be stressed that special care be taken after operation to prevent lethal tetany.

SUMMARY

The following points were noted in a patient suffering from extensive decalcification of the bones, combined with violent pain:

1. The illness began as osteitis fibrosa generalisata. Therefore there existed a pseudo-osteomalacic end-stage of Recklinghausen's disease.

2. There was a great increase in the calcium content of the serum. The deviation was considered to be a symptom of hyperfunction of the parathyroids.

3. There was a small swelling in the thyroid.

On account of these facts, a tumor of a parathyroid gland was suspected, and was found during operation. After extirpation of this small tumor, clinical recovery occurred.

15. Regnier: Fortschr. a. d. Geb. d. Röntgenstrahlen **39**:696, 1929.
16. Blumgart: Personal communication to the author.

EXPERIMENTAL GASTRIC ULCER

THE EFFECT OF THE CONSISTENCY OF THE DIET ON HEALING*

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Clinical experience and experimental observations indicate that the consistency of the diet may have a bearing on the healing of gastric ulcers.

That the physician believes the consistency of the diet is an important therapeutic item in the management of patients with ulcers is shown by the fact that a liquid or soft diet, with or without a preceding period of starvation, is used in all the generally accepted therapeutic procedures. Bolton,[1] in his book on "Ulcer of the Stomach," stated that in his opinion "there is no doubt that diet influences the production and propagation of ulcer of the stomach." He stated that "excessive amounts of imperfectly masticated, and hurriedly swallowed food of 'indigestible' quality, although alone being unable to produce the initial lesion, yet are able to assist in so doing, and in promoting the extension and delaying the healing of an ulcer of the stomach."

The fact that increased motility of the stomach associated with gastric retention delays the healing of experimentally produced acute ulcers of the stomach has been demonstrated by Bolton,[2] Friedman and Hamburger,[3] and Ivy,[4] who produced acute lesions of the stomach in dogs with partial pyloric stenosis, although all are agreed that partial stenosis per se does not give rise to the initial lesion.[5] The relative hyperacidity and hypernormal secretion associated with partial pyloric

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1. Bolton: Ulcer of the Stomach, New York, Longmans, Green & Company, 1913.

2. Bolton: Proc. Roy. Soc. Lond. **82**:236, 1909.

3. Friedman, J. C., and Hamburger, W. W.: Experimental Chronic Gastric Ulcer, J. A. M. A. **63**:380 (Aug. 1) 1914.

4. Ivy, A. C.: Contributions to the Physiology of the Stomach, Arch. Int. Med. **25**:6 (Jan.) 1920.

5. Ivy, A. C.; Droegemueller, E. H., and Meyer, J. L.: Effect of Experimental Pyloric Stenosis on Gastric Secretion, Arch. Int. Med. **40**:434 (Oct.) 1927.

stenosis may have been a contributing factor in delaying healing, but not a fundamental one when viewed in the light of Dragstedt's [6] finding that an acute ulcer produced in a gastric pouch so made as to retain its gastric secretion healed as quickly as an acute ulcer produced in a pouch in which the gastric juice was not retained. It was further shown by Ivy [7] that simple manipulation with the finger of an acute lesion of the mucosa of a pouch of the pyloric antrum, in the absence of acid, delayed the healing of the acute lesion. The same manipulation with a cotton pledget soaked in 0.4 per cent solution of hydrochloric acid has the same effect as manipulation, except that bleeding occurs earlier. This demonstrates that a mechanical factor can delay the healing of an acute lesion of the gastric mucosa, and that acid makes the ulcer more irritable or susceptible to bleeding.

It is an established fact that coarse particles of food remain in the stomach longer than fine particles, which obviously results in more prolonged motor activity of the stomach and increases the trauma to an acute lesion of the mucosa. This would be pronounced in a patient with pylorospasm.

Although the evidence indicates that the consistency of the diet has an important bearing on the healing of gastric ulcer, we have been unable to find any experimental work bearing directly on this problem.

Our attention was attracted by the work of Ferguson,[8] who was able by a certain procedure to produce in rabbits a lesion of the gastric mucosa which persisted for from two to eight months or longer, and which manifested the gross and histologic characteristics of a chronic ulcer. Since it is well known that the rabbit's stomach is filled constantly with oat hulls and very rough material, it occurred to us that we had at hand a method for studying the effect of the consistency of the diet on the healing of gastric ulcers.

METHODS FOR EXPERIMENTAL WORK

Ferguson's method is to expose the ventral wall of the stomach. An incision 3 or 4 cc. in length is made through the serosa and muscularis down to the mucosa in the region of the pyloric antrum. The mucosa is separated from the muscularis by undercutting, so that an area of mucosa 2 cm. in diameter is freed and exposed. This area of mucosa is then excised and the muscularis and serosa closed with a silk suture. This results in an ulcer which quite uniformly measures from 1.0 to 1.5 cm. in diameter, a statement we are able to make because a number of our rabbits died of pneumonia within a day or two after the operation.

6. Dragstedt, L. R.: Contributions to the Physiology of the Stomach, J. A. M. A. **68**:330 (Feb. 3) 1917.

7. Ivy, A. C.: Studies on Gastric and Duodenal Ulcer, J. A. M. A. **75**:1540 (Dec. 4) 1920.

8. Ferguson, A. N.: Cytological Study of Regeneration of Gastric Glands Following Experimental Removal of Large Areas of Mucosa, Am. J. Anat. **42**: 403, 1928.

In nine rabbits we produced ulcers 1.5 to 2 cm. in diameter on the dorsal or posterior wall of the stomach. In this operation it was necessary to push the contents of the pyloric antrum back into the fundus, where it was held by a low tension clamp during the period of the operation. The opening in the ventral or anterior wall made it possible to excise the piece of mucosa from the dorsal wall of the stomach; then the opening in the anterior wall was closed. The mortality from this procedure was fairly high, about 30 per cent due to intragastric hemorrhage, pneumonia or gastric paresis.

The rabbits were fed either a rough diet or a soft diet. The rough diet consisted of the usual stock ingredients; alfalfa hay, oats and raw carrots. The soft diet consisted of bread, milk and boiled carrots finely mashed. The rabbits to be placed on the soft diet were kept on that diet from one to two weeks prior to operation. We found that the stomach of these rabbits at the time of operation usually contained definitely less material than those fed on the hard diet, and the contents were more fluid in consistency. Some rabbits did not do well on this diet and died prematurely, that is, before the thirty day postoperative period had elapsed. The rabbits were killed after thirty days, and the stomach was examined for the presence or absence of an ulcer, its diameter being measured.

RESULTS

Simple Lesion on Posterior Wall.—In the nine rabbits in which a lesion was produced by excision of the mucosa of the posterior wall, all ulcers were healed in thirty days, five rabbits being fed on the soft diet and four on the rough diet.

In two of the four on the rough diet, an anterior as well as a posterior lesion was also produced. In these two rabbits the lesion on the posterior wall had completely healed, but in both cases the lesion on the anterior wall, which had been closed with silk, had developed into a chronic ulcer which had perforated into the liver.

These results show that the acute lesion of the posterior wall which contained no silk suture healed in spite of the consistency of the diet, and indicate that the silk suture plus the rough diet was the probable cause of the delayed healing.

In order to test this possibility further, in thirty-nine rabbits we made anterior lesions, closing them with a silk suture, nineteen being on a soft diet and fourteen on a rough diet.

On a Soft Diet With Anterior Lesion Plus Silk Suture.—Of the twenty-two rabbits used, seventeen lived through the thirty day test period, five dying prematurely at two, nineteen, eighteen, twenty-three and twenty-six days, all with ulcers that had not healed. Of the seventeen that were killed on the thirtieth day, only three had ulcers. The scars were easily found, and in some the scar showed a papillomatous-like overgrowth of the mucosa, as observed by Shapiro and Ivy [9] in their study of ulcer in the rabbit.

9. Shapiro, P. F., and Ivy, A. C.: Gastric Ulcer, Arch. Int. Med. **38**:237 (Aug.) 1926.

These results show that the silk suture per se is not the cause of the delayed healing noted in the foregoing and following experiments, because healing occurred on this diet with the silk encapsuled in situ in the scar.

On Rough Diet with Anterior Lesion Plus Silk Suture.—There were twelve rabbits with anterior lesions on the rough diet. When killed on the thirtieth day, all had typical chronic ulcers varying from 3 to 8 mm. in diameter. One rabbit that was killed at forty-two days showed complete healing. Others kept for longer periods than thirty days failed to show complete healing. Ferguson [10] recently showed us an ulcer made according to his method, the one we used, which had not healed in two years.

These results on the soft and rough diet with the anterior lesion show conclusively, we believe, that the consistency of the diet is an important factor in determining the healing time of the ulcer, provided that an additional factor (the silk suture or connective tissue factor) tending to delay healing is present. That the rough diet is not the sole factor is shown by the fact that the simple acute ulcer on the posterior wall (without the silk) healed in thirty days, irrespective of the consistency of the diet. These results also demonstrate that the reason why Ferguson was able to obtain chronic ulcers consistently by his method was that he used a rough diet in addition to the silk suture, which is not absorbed but encapsuled by connective tissue.

The fact that the amount of healing on the rough diet varies from one rabbit to another, is best explained on the basis of a difference in the growth capacity of the fibroblasts and mucosal cells in the different rabbits, rather than on the basis of the variation in the size of the acute lesion. A faster rate of growth and maturity of the connective tissue elements would reduce the blood supply to the more slowly growing and more highly differentiated mucosal cells and hence make it more difficult for the mucosal cells to cover the defect. The silk, being nonabsorbable and acting as a foreign body, excites fibroblastic reaction. In the absence of this excessive connective tissue growth, the ulcer heals irrespective of the diet. The observations of Dragstedt and Vaughn [11] on the production of chronic gastric ulcer in the dog by taking a number of stitches with silk in the base of an acute ulcer further supports this explanation, especially since in some dogs, in spite of the suture, the ulcer healed, while in others it became chronic. The rough diet, with the associated augmented motility, renders it difficult for the mucosal cells to obtain a foothold on the base of the ulcer and

10. Ferguson, A. N.: Personal communication to the author.

11. Dragstedt, L. R., and Vaughn, A. M.: Gastric Ulcer Studies, Arch. Surg. 8:791 (May) 1924.

to establish permanent relations with the underlying capillaries which is their source of nourishment; in other words, we believe that factors which tend to disturb the blood supply or nutrition of the proliferating mucosal cells are responsible for delaying healing.

Rôle of Gut Suture Versus Silk Suture.—It is well known that a silk suture is not absorbed but is encapsulated by connective tissue; whereas a gut suture, although it causes some foreign body reaction, is finally absorbed or causes less connective tissue proliferation. Because of this difference an ulcer was produced in the anterior wall, the

Fig. 1 (rabbit 51).—A three day lesion showing the type of lesion produced; this one measured 8 by 10 mm.

incision was closed with gut instead of silk, and the rabbits were placed on a rough diet. This was done in four rabbits, with the result that in three of the four, typical ulcers were present. In the other, almost complete healing (no pitting, ulcer defect 1 by 2 mm.) had occurred. These results show that the gut suture caused sufficient connective tissue proliferation or a sufficient amount of some unknown disturbance to delay healing. They do not prove, however, that no difference exists between gut and silk in delaying healing; to settle this point, more rabbits must be used and a longer postoperative period allowed as a test period.

Fig. 2.—Ulcer 7 by 7 mm., silk suture on the anterior wall, which perforated into the liver in rabbit on rough diet.

Fig. 3 (rabbit 22).—Ulcer 6 by 6 mm., silk suture on the anterior wall of rabbit on rough diet.

Fig. 4 (rabbit 66).—Ulcer 10 by 10 mm., silk suture on the anterior wall of a rabbit on diet of dry rolled oats.

Fig. 5 (rabbit 19).—Ulcer 8 by 8 mm., gut suture on the anterior wall of a rabbit on a rough diet.

Specific Qualities of the Diet.—Other factors than the consistency of the diet must be considered, such as the fat content which inhibits the formation of gastric secretion during the early part of the digestive period and the buffer value of the food, or the capacity the food possesses of combining with free acid, and roughage.

We have not as yet performed experiments to analyze the importance of all these factors, but we have performed an experiment relative to the roughage factor.

Four rabbits were placed on a diet consisting solely of Quick Rolled Oats in the dry form, which avoided the roughage from the oat hulls, hay and lumps of raw carrots. An ulcer was produced in the anterior gastric wall as described, the defect in the muscularis being closed with silk. When the stomach of these rabbits was opened, the contents were found to be as pasty and as dry as that found in the rabbits fed

Fig. 6 (rabbit 66).—Photomicrograph of ulcer in figure 4. The steep edge of the ulcer was the edge nearest the cardia; × 6.

on the oats and hay diet. An ulcer was found in all four rabbits when they were killed at the thirty day period. This shows that a high percentage of roughage alone is not the only factor operating in the rough diet, and that the fluidity of the gastric contents is a factor which must be considered as important.

Other experiments are under way to test further the fluidity, fat and food buffer factors.

COMMENT

Every investigator is agreed that simple or uncomplicated acute lesions of the gastric mucosa of animals heal rapidly and almost without failure. Those of us who have done considerable gastric analysis on normal man, withdrawing the contents by suction, have not infrequently observed streaks of blood and pieces of mucosa in the withdrawn fluid, and without changing the diet in any way have not

observed a chronic ulcer to occur. The foregoing statement for animals is true to some extent, at least, for man. However, given a man with a chronic ulcer, or a man with a lesion of the stomach plus the factor or factors responsible for the chronicity, our results from experiments on the rabbit indicate that a soft diet would facilitate the healing of the gastric lesion.

SUMMARY

A simple ulcer of the rabbit's stomach (1.5 to 2 cm. in diameter) produced by excision heals within thirty days, irrespective of the consistency of the diet.

Healing of Acute Lesion of Gastric Mucosa As Influenced by a Rough and Soft Diet

Rough Diet			Soft Diet		
Rabbit Number	Days Post-operative	Ulcer Size in Mm.	Rabbit Number	Days Post-operative	Ulcer Size in Mm.
17	30	1.5 × 3	38	31	Complete healing
22	30	6 × 6	5	30	2 × 6
10	30	6 × 7	25	30	Complete healing
18	30	2 × 3	42	30	Complete healing
51	30	1.5 × 3	47	30	Complete healing
67	30	2 × 3	77	31	2 × 4
8	30	8 × 10	76	30	Complete healing
1	30	5 × 9	24	30	Complete healing
4	30	3 × 4	26	30	Complete healing
88	30	1 × 2	74	30	Complete healing
25	30	3 × 4	85	30	Complete healing
73	30	5 × 6	71	30	Complete healing
100	42	Complete healing	34	30	1.5 × 3
			55	30	Complete healing
			97	30	Complete healing
			78	32	Complete healing
			44	30	Complete healing
			89	19	3 × 4
			80	18	7 × 10
			7	2	15 × 20
			6	23	2 × 5
			61	26	2 × 3

A similar ulcer, but with a silk suture in its base, will heal if the rabbit is fed a soft diet, but will tend to become chronic if the rabbit is fed a rough diet. We have confirmed Ferguson's [8] observation that if an acute lesion is made by his method and the rabbits are kept on a rough diet, a chronic ulcer results in numerous instances.

The consistency of the diet has an influence on the healing of an ulcer of the stomach, if other factors that tend to delay the healing are operating simultaneously.

These results indicate that in chronic ulcer in man, a soft diet would facilitate the healing of the ulcer, which confirms clinical experience and supports the use of a diet of the character of that employed in the generally accepted therapeutic procedures for gastric ulcer.

THE LIFE OF RETICULOCYTES

EXPERIMENTS ON THEIR MATURATION *

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The life history of red blood corpuscles has fascinated many investigators. A large amount of literature has accumulated regarding the development of red blood corpuscles, one phase of which concerns cells containing basophilic substance or, when supravitally stained, reticular substance and commonly known as reticulocytes. Since Theobald Smith [1] first suggested that these were young cells rather than degenerative forms of the adult red blood cell, an abundant amount of evidence has been presented which indicates clearly that the basophilic substance is a sign of youthfulness.

Some of the evidence that reticulocytes are less mature than nonbasophilic erythrocytes is well founded on multiple observations in states of anemia in which an increase of reticulocytes in the peripheral circulation precedes an increase in nonreticulated erythrocytes. Nevertheless, little information is available concerning the fate of such young cells: the duration of their stay in the circulation, the disposal of the basophilic substance and whether they develop into nonbasophilic erythrocytes or are destroyed. Without doubt, under normal conditions in adult persons any maturation of the majority of these cells must take place in the bone-marrow. Indirect evidence favors the opinion that reticulated cells which reach the circulation remain for a few days and mature there. Pepper [2] observed that reticulocytes in rabbits' blood kept in the incubator gradually diminished in number and disappeared by the third day. Morawitz and Denecke [3] stated that if blood is allowed to age in vitro, these cells disappear after from one to two days.

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* From the Thorndike Memorial Laboratory of the Boston City Hospital and the Department of Medicine of the Harvard Medical School.

1. Smith, Theobald: On Changes in the Red Blood-Corpuscles in the Pernicious Anemia of Texas Cattle Fever, Tr. A. Am. Phys. **6**:263, 1891.

2. Pepper, O. H. P.: Observations on Vitally Stainable Reticulation and Chromatic Granules in Erythrocytes Preserved in Vitro, Arch. Int. Med. **30**:801 (Dec.) 1922.

3. Morawitz, P., and Denecke, G.: Handbuch der inneren Medizin, Berlin, Julius Springer, 1926, vol. 4, p. 53.

These authors, however, do not suggest this as evidence in regard to the duration of the life of reticulocytes in the blood stream. Seyfarth and Jürgens [4] noted that the cells with "substantia granulofilamentosa" gradually diminished in the blood stream of mouse embryos with increasing age of the embryos and continued to diminish after birth. Cohn, and Minot and their collaborators [5] suggested from their observations on the reticulocyte response in pernicious anemia following liver therapy, that reticulocytes in the blood may not lose the reticular material for from five to ten days.

Observations concerning the direct effect of liver extracts, which are capable of producing complete remission in pernicious anemia, on the maturation of reticulocytes in vitro have been essentially negative.[6] These observations led to a study of the changes in the number and appearance of the reticulocytes under certain experimental conditions. The results of this study had a direct bearing on the life of reticulocytes after they enter the blood stream and are presented here.

METHODS

Blood containing large numbers of reticulocytes was obtained from two sources: (1) human patients, especially those with cases of pernicious anemia at the peak of the reticulocyte rise following liver therapy, and (2) rabbits that either had been bled from the ear every other day or had been given phenylhydrazine intraperitoneally. The blood was collected under sterile precautions, placed in a small sterile flask containing glass beads and defibrinated by gentle shaking. Usually 0.5 cc. of defibrinated blood was placed in a small sterile test tube, corked and placed in an incubator at 37 C. As a rule, five such preparations were used for each blood to be tested.

Smears of blood were made on glass cover-slips and stained supravitally with brilliant cresyl blue and counterstained with Wright's stain after the methods described by Hawes [7] and Cunningham.[8] Thus permanent preparations of stained reticulocytes were available for prolonged observation and future reference. Usually, 1,000 erythrocytes were counted, areas on both cover slips being used, and the reticulocyte percentage was determined.

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4. Seyfarth, C., and Jürgens, R.: Untersuchungen über das Verhalten der vitalgranulierten roten Blutzellen (Retikulocyten) bei Embryonen und Neugeborenen, Virchows Arch. f. path. Anat. **266**:676 (Jan.) 1928.

5. Cohn, E. J.; Minot, G. R.; Alles, G. A., and Salter, W. T.: The Nature of the Material in Liver Effective in Pernicious Anemia: II, J. Biol. Chem. **77**:325 (May) 1928.

6. Heath, C. W., and Daland, G. A.: The Basophilic Substance of Young Red Blood Cells: The Influence of Solutions of Different Substances upon the Supravital Staining with Brilliant Cresyl Blue, to be published.

7. Hawes, J. B.: A Study of Reticulated Red Blood Corpuscles by Means of Vital Staining Methods, Its Relation to Polychromatophilia and Stippling, Boston M. & S. J. **161**:493 (Sept.) 1909.

8. Cunningham, T. D.: A Method for the Permanent Staining of Reticulated Red Cells, Arch. Int. Med. **26**:405 (Oct.) 1920.

DECREASE OF RETICULOCYTES IN VITRO AT 37 C.

A study of table 1 shows that at 37 C. the number of cells with reticular substance diminishes gradually over the course of three or four days. During the first few days the cells remained in good condition. At the end of four days, especially in the case of rabbits' blood, there was a tendency to hemolysis, sometimes marked, and the red cells tended to become distorted, so that further observations were not relia-

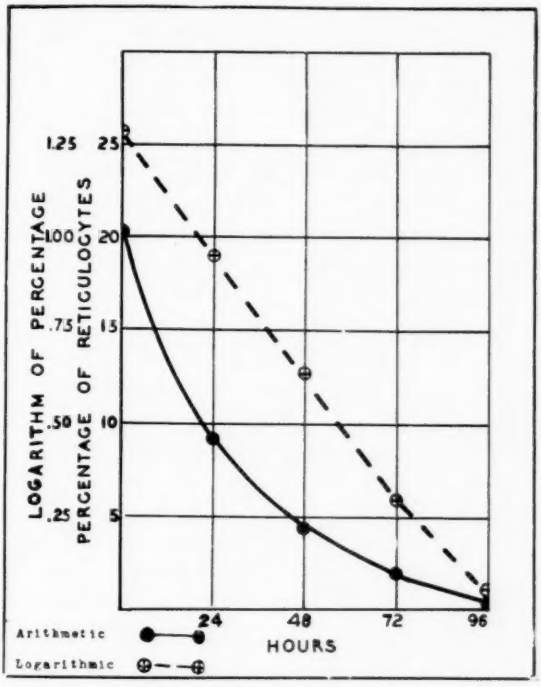

Fig. 1.—A typical example of the rate of decrease in the number of reticulocytes in vitro at 37 C. over a four day period.

ble. This decrease of reticulocytes at 37 C. is absolute as well as relative, because the total red blood cell counts remained constant within the limits of error during the first seventy-two hours. There is a variation in the rate of disappearance of these cells in the different experiments, but there is no fundamental difference to be observed between blood coming from different sources, e. g., between the rabbit and the human being, between bled rabbits and those into which injections of phenylhydrazine were made and between cases of hemolytic jaundice

and those of pernicious anemia. Therefore, it may be assumed that, at least in this regard reticulocytes are of the same general nature no matter what the cause inciting their increased output into the circulation.

If the results of any of these experiments are plotted with the percentages of reticulocytes as ordinates and the period of time as abscissas, as in figure 1 (experiment 16, table 1), there results a typical exponential curve. If this curve is plotted with the logarithms of the reticulocyte percentages as ordinates, the result is a straight line. This type of curve is typical of many simple biologic and chemical phenomena, and is characteristic of senescent phenomena.

TABLE 1.—*The Decrease of Reticulocytes in Vitro at 37 C.*

Diagnosis	Experiment	Patient	Time Relative to Peak of Reticulocyte Response	Percentage of Reticulocytes*					
				Start Before Incubation	24 Hrs. After Incubation	48 Hrs. After Incubation	72 Hrs. After Incubation	96 Hrs. After Incubation	120 Hrs. After Incubation
Pernicious anemia	1	Ty	1 day before	33.3	17.5	7.3	3.8	...	...
	2	Ty	1 day after	29.5	9.3	6.3	3.2	...	...
	3	Ld	1 day before	22.1	8.6	6.3	3.5	...	...
	4	Br	2 days before	23.3	18.1	15.1	12.3	5.7	...
	5	Br	Day of peak	42.1	27.6	20.5	14.0	...	5.6
	6	Br	2 days after	34.5	21.0	13.0	6.7	2.2	...
	7	Cn	2 days after	15.2	3.3	2.0	0.8	...	...
	8	Gn	1 day before	26.7	14.4	9.7	...	3.1	...
	9	Gn	Day of peak	36.8	11.5	....	3.7	...	1.1
	10	Ky	1 day before	28.2	17.6	8.5	4.6	1.9	...
Purpura hemorrhagica	11	Wx	.....	26.2	15.3	11.3	4.8	0.8	...
	12	....	.....	17.3	7.8	4.4	3.1	0.7	...
Chronic familial hemolytic jaundice	13	Ce	.....	10.8	3.5	1.5	0.7	...	...
	14	Yk	.....	39.2	17.7	11.4	8.0	...	...
Normal	15	Hh	.....	0.6	0.5	....	0.0	...	...
Rabbits rendered anemic by bleeding	16	R_1	.....	32.5	9.6	5.4	...	...	...
	17	R_2	.....	20.1	9.0	4.4	2.0	0.4	...
	18	R_3	.....	18.6	5.6	4.8	1.6	0.2	...
Rabbits into which injections of phenylhydrazine were made	19	R_4	.....	21.9	9.6	9.4	5.1	...	...
	20	R_5	.....	24.3	9.8	8.4	...	...	...
	21	R_6	.....	19.5	11.5	7.6	...	...	...
	22	R_7	.....	30.3	18.6	14.8	4.8	...	...

* The results reported are the averages for the percentages of reticulocytes from five tubes in all experiments with the following exceptions: 18 and 21, four tubes; 14, three tubes; 10 and 8, two tubes; 15 and 22, one tube each.

DECREASE OF RETICULOCYTES IN THE PLEURAL CAVITY OF THE RABBIT

In order to place the cells under more normal physiologic conditions, from 8 to 10 cc. of defibrinated blood rich in reticulocytes was injected into the pleural cavity of rabbits by the following technic: The skin over the back in the lower thoracic region was shaved, procaine hydrochloride was injected and the pleura was exposed between the seventh and eighth ribs under sterile precautions. At varying periods over the course of two or three days, a small needle was inserted into the eighth or ninth interspace, and a drop or two of the previously injected blood was with-

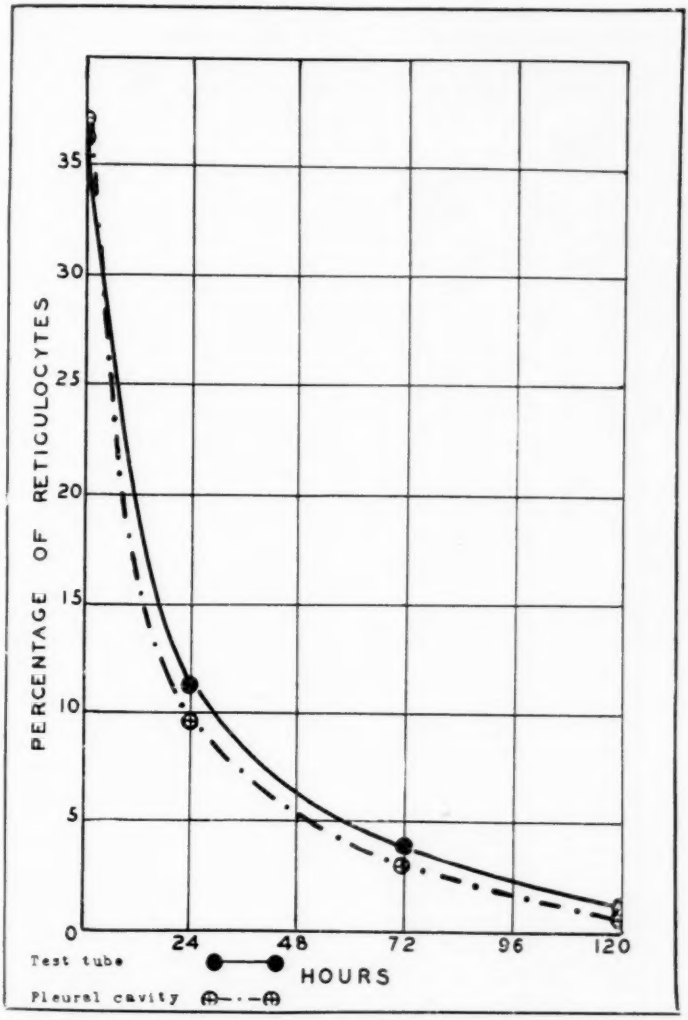

Fig. 2.—A comparison of the rate at which reticulocytes decrease in the test tube at 37 C. with the rate at which they decrease in the pleural cavity of the rabbit.

drawn from the pleural cavity. Usually, after two or three days none of the injected blood could be obtained. Autopsy at this time revealed only a small amount of blood moistening the walls of the pleural cavity. The viscosity of this blood seemed much increased. Smears of the blood showed many large, vacuolated, phagocytic cells. The red blood cells were relatively uninjured, and the reticulocytes stained as well as after being in the incubator for several days.

Figure 2 illustrates the decrease of reticulocytes of the blood from a case of pernicious anemia (experiment 9, table 1). Samples were placed in the pleural cavity of a rabbit and at the same time in test tubes which were incubated at 37 C. The curves show a striking similarity between the decrease of reticulocytes in the incubator and that in the pleural cavity of the rabbit.

Five experiments of this nature were performed with rabbit's blood, with the result that the amount of reticulocytes decreased at a rate similar to that of reticulocytes in the incubator (table 1). No especial

TABLE 2.—*The Decrease of Reticulocytes at Room Temperature and in the Icebox*

Experiment	Temperature, C.	Percentage of Reticulocytes					
		Start	24 Hrs.	48 Hrs.	72 Hrs.	96 Hrs.	120 Hrs.
18	37	21.9	9.6	9.4	5.1	....	....
	23	22.4	20.1	....	21.0	....	23.0
21	37	30.3	18.6	14.8	4.8	....	....
	10	28.4	29.4	28.2	27.4	25.2	26.4

advantage of this method was demonstrated and it was abandoned. Phagocytosis of the red blood cells apparently was active in the pleural cavity, but reticulocytes were no more rapidly taken up than adult red blood cells. Evidence of phagocytosis in smears of blood from the test tube experiments has been observed only rarely.

THE DECREASE OF RETICULOCYTES IN VITRO AT TEMPERATURES LOWER THAN 37 C.

Similar test tube preparations of blood with a high reticulocyte count were kept in an icebox (10 C.) or at room temperature (23 C.) and observed over long periods. Reticulocytes could still be demonstrated at the end of six months when kept in the icebox. Pepper [2] observed reticulocytes in blood preserved in the icebox for one month, and under similar circumstances Key [9] observed them for sixteen days.

In table 2 are recorded two of the numerous observations on rabbit's reticulocytes kept at temperatures lower than 37 C. There is no definite

9. Key, J. A.: Studies on Erythrocytes, with Special Reference to Reticulum, Polychromatophilia and Mitochondria, Arch. Int. Med. **28**:511 (Nov.) 1921.

evidence that there was any decrease over five or six days. Many of the reticulocytes developed small granules, taking the brilliant cresyl blue stain after being in the icebox for a few days. This phenomenon will be described presently. Lowering the temperature inhibits the decrease of reticulocytes which takes place so actively at 37 C.

THE RELATION OF THE DECREASE OF RETICULOCYTES TO DEGENERATIVE CHANGES OF BLOOD IN VITRO

Although hemolysis of the red blood cells was slight when blood was incubated for a few days and occurred much more slowly when blood was kept in the icebox, it is possible that the liberation of hemoglobin may have a significant influence on the decrease of reticulocytes in vitro. However, this seems unlikely. Pepper[10] summarized the inconsistent statements in the literature concerning the fragility of reticulocytes, and he was unable to demonstrate that reticulocytes are more or less fragile to hypotonic salt solutions than adult red blood cells. There is no conclusive evidence that reticulocytes are more easily destroyed than non-reticulocytes, and, in fact, there is some evidence that at least under certain conditions they are more resistant.[11]

In the pleural cavity of the rabbit, while active phagocytosis was taking place, it was observed that the reticulocytes of the injected blood decreased at a similar rate to those in the blood incubated in test tubes. Therefore, reticulocytes are no more easily destroyed by phagocytosis than adult red blood cells.

Whereas there is only a very slight trace of hemolysis in blood incubated for twenty-four hours, the greatest decrease of reticulocytes takes place during this period. Repeated red blood cell counts of the incubated specimens performed during the first forty-eight hours varied within the limits of technical error. After seventy-two hours there was a slight increase in the number of shadow cells seen in the counting chamber but these numbered only 2 or 3 per cent of the total count, whereas the decrease in the number of reticulocytes was of a much greater magnitude. If distilled water, up to 0.8 cc., was added to 1 cc. of whole blood, there was no definite decrease in the reticulocyte percentage in spite of definite hemolysis. When equal parts of distilled water and blood were mixed, there was about a 50 per cent reduction of the number of reticulocytes, but the reduction of the total number

10. Pepper, O. H. P., and Peet, M. M.: The Resistance of Reticulated Erythrocytes, Arch. Int. Med. **12**:81 (July) 1913.

11. Isaacs, R.; Brock, B., and Minot, G. R.: The Resistance of Immature Erythrocytes to Heat, J. Clin. Investigation **1**:425 (June) 1925. Minot, G. R., and Buckman, T. E.: Erythremia (Polycythemia Rubra Vera), Am. J. M. Sc. **166**:469 (Oct.) 1923.

of red blood cells was proportionately much greater. These facts seem to indicate that slight hemolysis, such as occurred in some of the test tube specimens, is not a factor in influencing the results of these experiments. The discrepancies in the reticulocyte percentage decrease which occur after incubation for ninety-six hours may be due to extreme hemolysis.

A change in hydrogen ion concentration, which may accompany autolytic changes in incubated blood, perhaps may influence the reticulocytes. Gawrilow [12] found that slight changes in the hydrogen ion concentration affected the staining of reticulocytes by polychrome-methylene blue. This has not been found to be true for the method of staining reticulocytes used in this laboratory. Indicator tests of the p_H values of the supernatant serum after incubation of the blood for twenty-four and forty-eight hours have shown no significant change. Controlled experiments have been performed with cells in contact with large and small amounts of serum; this serum has been drawn off and replaced by fresh serum several times a day, without changing the rate at which the amount of reticulocytes decreased. Because of the possibility that there may be local changes about cells which rest compactly in the bottom of a test tube, blood has been automatically shaken in the incubator for several days, but this procedure also had no definite influence on the rate of decrease of reticulocytes. Cells placed in contact with buffer solution, salt solution and solutions of glycine, alanine or liver extract, which had different p_H values than serum, showed no constant difference in the rate of decrease of the amount of reticulocytes.

It appears, therefore, that the decrease in the number of reticulocytes in vitro is a phenomenon which occurs irrespective of degenerative changes taking place in blood in vitro.

THE TRANSFUSION OF RETICULOCYTES

A series of experiments was performed in which citrated rabbit's blood containing from 20 to 30 per cent of reticulocytes was transfused into normal rabbits. In all cases there was an immediate rise of the reticulocyte percentage in the peripheral blood, usually to more than twice the initial count, or from about 3 to 6 per cent. Within from twelve to forty-eight hours, the reticulocyte percentage dropped slowly toward the initial level. No dependable conclusion regarding the maturation rate of reticulocytes can be drawn from these experiments because the reticulocyte count of the rabbit varies considerably from time to time under normal conditions, and also because a relatively large

12. Gawrilow, R.: Zur Lehre über die vitalfärbbare Substanz der Erythrozyten, Folia haemat. **38**:216 (June) 1929.

error is introduced in counting reticulocytes when they are few in number. The influence of transfusion itself on the reticulocyte level is also a factor. The results of the experiments, however, are not inconsistent with the rate of decrease in the number of reticulocytes in incubated blood or in blood placed in the pleural cavity, and they indicate that reticulocytes injected into the circulation are not immediately withdrawn; for example, by phagocytosis or by some organ.

THE APPEARANCE OF GRANULES IN THE RED BLOOD CELLS

Small granules in many of the reticulocytes, and in cells free from reticular substance, have been observed in both human and rabbit blood, after incubation and after remaining in the icebox. These granules are more pronounced in the cells of the human blood that was studied than

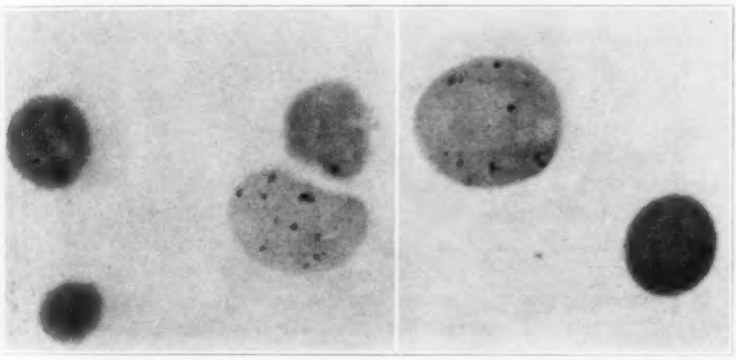

Fig. 3.—Basophilic granules in the red blood cells incubated at 37 C. for forty-eight hours. Wright's stain; × 2,200. The blood was taken from a case of pernicious anemia at the peak of the reticulocyte response following liver therapy.

in the cells of the rabbit blood. In cells without reticular substance the granules number usually from one to twenty, and sometimes even more. They appear scattered through the cell; sometimes one or two may be at the periphery. These granules vary in size, are often irregular in shape and stain with brilliant cresyl blue and Wright's stain (fig. 3). Sometimes they appear as short rods resembling what Schilling [13] has described as "erythrokonten." Basophilic rods resembling Schilling's "erythrokonten" have been observed in the unincubated blood from cases of pernicious anemia, hemolytic jaundice and purpura hemorrhagica.

13. Schilling, V.: "Erythrokonten," Stäbchen in Erythrocyten, bei Anaemia perniciosa und einigen nahestehenden Krankheitsbildern, Klin. Wchnschr. **7**:785, 1928.

Pepper [2] observed similar granules in rabbit's blood kept on ice. They undoubtedly are not the "chromatic granules" which he observed in moist preparations of incubated rabbit's blood and which he related to the "substantia metachromaticogranulosa" of Cesaris-Demel.[14] The granules do not resemble Howell-Jolly bodies, or the nuclear particles described by Morris,[15] and they cannot arise from the nucleus in bloods which contain very few or no nucleated erythrocytes. Dr. Raphael Isaacs examined some of the preparations showing these granules, and he stated that they were not identical with the granules described by him.[16] They were seen in cells from cases of pernicious anemia at the height of the reticulocyte response following liver therapy. In a case of chronic familial hemolytic jaundice, 6 per cent of the red blood cells contained such granules. In the reticulocytes of blood incubated for forty-eight hours, these granules appear in the midst of the reticular substance; sometimes they are present in greater amount than the reticular substance itself.

The cells containing granules but not reticular substance (that is, strands or net forms staining with brilliant cresyl blue) were counted in blood incubated as usual for from three to four days. These counts were compared with the reticulocyte counts of the same preparations. Figure 4 gives data from a case of pernicious anemia in which a comparison is made between the number of cells containing granules only and the number of cells containing reticular substance. Whereas the number of reticulocytes decreased on incubation of the blood, the number of cells containing granules increased, and subsequently decreased at a slower rate than the reticulocytes. The maximum number of cells with granules appeared at different times in the experiments on three different days. The significance of this observation will be discussed later.

Similar but less pronounced results were obtained with rabbit's blood containing large numbers of reticulocytes. Normal human blood, with 0.6 per cent reticulocytes, showed only 0.3 per cent of cells with granules after seventy-two hours of incubation.

These granules resemble individually the granules in punctate basophilia and the nodal points of the reticular substance seen when stained with brilliant cresyl blue. Punctate basophilia is probably of the same

14. Cesaris-Demel: Studien über die roten Blutkörperchen mit den Methoden der Färbung in frischen Zustande, Folia haemat. **4**:1 (Oct.) 1907.

15. Morris, R. S.: Nuclear Particles in the Erythrocytes, Arch. Int. Med. **3**:93 (March) 1909.

16. Isaacs, R.: The Refractive Granule Red Blood Corpuscle: Its Behavior and Significance, Anat. Rec. **29**:299, 1925.

nature as diffuse basophilia which manifests itself as reticular substance when stained supravitally by certain dyes. Many authors describe punctate basophilia as basophilic substance altered by the action of certain toxic substances such as lead.[17] Because of the appearance of these granules in reticulocytes and their increase with the decrease of the reticulocytes, it is logical to conclude that they arise from or are a form of the basophilic substance of young red blood cells. Their presence in freshly drawn human blood containing large numbers of reticulocytes makes it probable that they appear in vivo in the course of the development of the red cell.

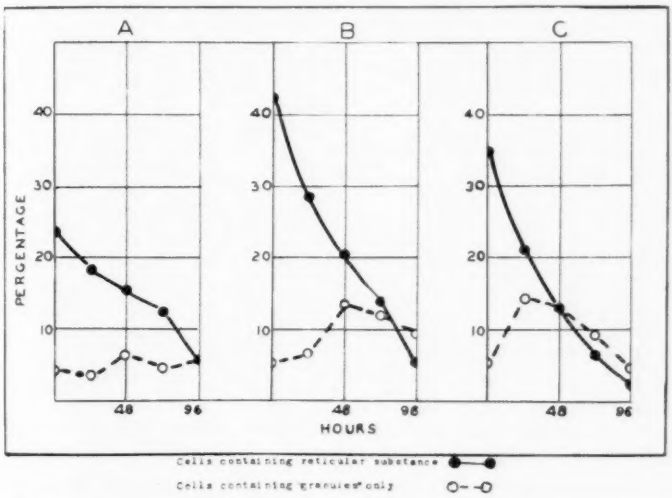

Fig. 4.—Progressive changes observed, in vitro, at 37 C. in the erythrocytes from a case of pernicious anemia during the reticulocyte response. *A*, two days before the peak of the reticulocyte response; *B*, at the peak of the reticulocyte response; *C*, two days after the peak of the reticulocyte response.

THE RELATION BETWEEN THE AGE OF THE RETICULOCYTES AND THEIR RATE OF DECREASE

Older cells should reach maturity in a shorter time than younger cells. If it can be shown that older reticulocytes decrease faster than younger reticulocytes in the incubator, the result will suggest that what

17. Pappenheim, A.: Spezielle Morphologie und Genese der Blutzellen, Folia haemat. **24**:110, 1919. Brookfield, R. W.: Blood Changes Occurring During the Course of Treatment of Malignant Disease by Lead, with Special Reference to Punctate Basophilia and the Platelets, J. Path. & Bact. **31**:277, 1928. Morawitz and Denecke (footnote 3). Gawrilow (footnote 12.)

occurs in vitro bears a close relation to processes of maturation in the blood stream.

It is exceedingly probable that younger reticulocytes contain larger amounts of reticular substance than older reticulocytes. The significance of different types of reticulocytes has been commented on by Lee, Minot and Vincent.[18] Seyfarth and Jürgens[4] studied the blood of mouse embryos and noted that with the increasing age of the embryos the number of red blood cells with a "thick, knotted form of substantia granulofilamentosa" diminished, while that of the cells with "speckled or net form" and cells without basophilic substance increased.

Gawrilow[19] divided reticulocytes into cells of three ages: the youngest are those with heavily constructed reticular substance; an intermediate type is recognized, and the most mature cells are those with isolated threads or granules. In anemia produced in guinea-pigs by the injection of phenylhydrazine, he found that as the number of reticu-

TABLE 3.—*Types of Reticulocytes in a Case* of Pernicious Anemia During the Reticulocyte Response*

Time Relative to Peak of Reticulocyte Response	Cells Containing Reticular Substance		
	Cells with Large Amount, per Cent	Cells with Medium Amount, per Cent	Cells with Small Amount, per Cent
Two days before peak	28.5	54.0	17.5
Day of peak	13.0	64.0	23.0
Two days after peak	6.5	60.5	33.0

* Table 1, experiments 4, 5 and 6.

locytes increased they were mostly of the heavily constructed type; at the peak of the rise the majority were of the intermediate type; later, most of them contained only isolated threads or granules.

In several of the cases of pernicious anemia, before, during and after the reticulocyte response due to liver therapy, a quantitative differential count of the reticulocytes was made. The reticulocytes were classified according to three types, those having large amounts of reticular substance, medium amounts and small amounts. All three types are present at the same time, but early in the reticulocyte response more cells have large amounts of reticular substance, whereas later, more cells have small amounts of reticular substance. In table 3 are given the percentages of cells in each of these three classes from the blood of a patient with pernicious anemia two days before the peak of

18. Lee, R. I.; Minot, G. R., and Vincent, B.: Splenectomy in Pernicious Anemia, J. A. M. A. **67**:719 (Sept. 2) 1916.

19. Gawrilow, R.: Experimentelle Untersuchungen über das Verhältnis verschiedener Retikulozytenformen zueinander bei akuten Anämien, Folia haemat. **38**:246 (June) 1929.

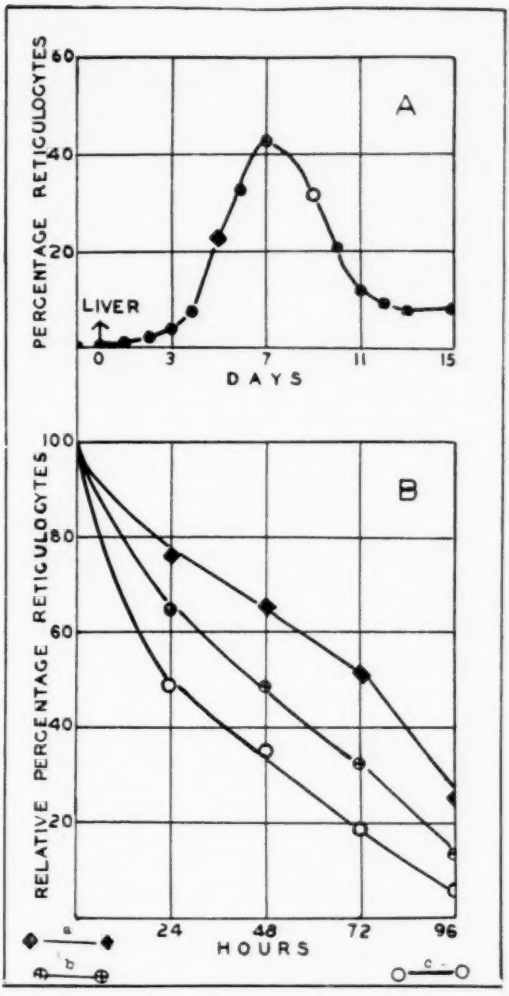

Fig. 5.—A study of the reticulocytes from a case (table 1, experiments 4, 5 and 6) of pernicious anemia during the reticulocyte response to liver therapy. Initial red blood cell count: 1,000,000 per cubic millimeter. *A* shows the percentage of reticulocytes in the blood of the patient following treatment. In *B* the blood from this patient, taken two days before the peak (*a*), at the peak (*b*) and two days after the peak (*c*), was incubated at 37 C. in test tubes. For purposes of comparison the initial level of the reticulocyte percentage for each experiment is considered as 100 per cent.

the reticulocyte response, at the peak and two days after the peak. Figure 5 *A* shows the reticulocyte response of this patient and records at what three times the quantitative differential count of the reticulocytes was determined.

At the same times blood was also taken for incubation at 37 C. The rate of decrease of the reticulocytes in vitro was determined for each of the three days and is shown on figure 5 *B*. They decreased in vitro slowest when they were increasing in the peripheral blood, or when the majority of the cells had large amounts of reticular substance. Furthermore, they decreased fastest in vitro when the majority of the cells had small amounts of reticular substance, or when the number of reticulocytes in the patient's blood stream had begun to decrease. In addition, there seemed to be a definite relationship between the rate of decrease of reticulocytes in incubated blood and the rate at which cells containing granules increased (fig. 4).

TABLE 4.—*Three Types of Reticulocytes from Blood* of Patients with Pernicious Anemia in Vitro at 37 C.*

Period of Incubation at 37 C.	Cells Containing Reticular Substance		
	Cells with Large Amount, per Cent	Cells with Medium Amount, per Cent	Cells with Small Amount, per Cent
Start	28.5	54.0	17.5
24 hours	12.5	59.0	28.5
48 hours	5.5	50.0	44.5
72 hours	4.0	51.0	45.0
96 hours	1.0	36.0	63.0

* Table 1, experiment 4.

A similar quantitative division of the reticulocytes was performed on many bloods incubated at 37 C. During the period of incubation and while the total number of reticulocytes was diminishing, the percentages of reticulocytes containing large amounts of reticular substance decreased while those containing small amounts increased. Furthermore, while reticulocytes were disappearing from the blood in vitro the reticular substance of those remaining was changing to a more mature form. An observation of this sort is given in table 4.

If blood is centrifugated, the reticulocytes tend to accumulate in the top layers of cells. This knowledge was utilized to separate the less mature reticulocytes, that is, those containing large amounts of reticular substance, from the more mature reticulocytes or those containing small amounts of reticular substance.

Table 5 gives data which show that there is an accumulation of reticulocytes in the upper portion of a column of cells. In addition, there is a slightly greater percentage of reticulocytes containing large amounts of reticular substance in the upper than in the lower part of the centrifugated specimen. Studies of the rate of decrease in vitro of

reticulocytes taken from the three layers of centrifugated blood show what is to be expected; namely, a slightly faster rate of decrease in the number of reticulocytes in the specimens containing particularly cells with smaller amounts of reticular substance than in those containing larger amounts of reticular substance.

These experiments seem to indicate a direct relation between the age of the reticulocytes and the period of time taken to reach maturity. In other words, reticulocytes probably reach maturity, or become nonreticulated erythrocytes, in a period of time depending on their age.

THE MATURATION OF RETICULOCYTES IN PERNICIOUS ANEMIA

The reticulocyte response in pernicious anemia following liver therapy has aroused a great deal of interest. The following question is to be answered: Do the reticulocytes which enter the circulation in great numbers develop into mature erythrocytes, or are they destroyed, for example, as foreign bodies?

TABLE 5.—*The Three Types of Reticulocytes in a Centrifugated Specimen of Blood*

Layer of Cells	Reticulocytes, per Cent	Cells Containing Reticular Substance		
		Cells with Large Amount, per Cent	Cells with Medium Amount, per Cent	Cells with Small Amount, per Cent
Top	38.4	28	49	23
Middle	9.4	21	51	28
Bottom	7.0	19	60	21

After the institution of liver therapy the reticulocytes usually begin to increase in number in from three to eight days and reach a maximum in the subsequent three to six days.[20] Minot and Cohn and their collaborators [20] suggested that the total erythrocyte increase up to the peak of the reticulocyte rise may be accounted for largely by the increase in these young cells. Following this, the total red cells count increases while the reticulocyte count decreases, probably because mature red cells are entering the circulation and because reticulocytes are maturing in the circulating blood.

The course taken by the reticulocytes after the institution of liver therapy depends somewhat on the amount and rate at which the active principle is administered and on the precise state of the given patient. Within certain limits, if large amounts of the active principle are fed, the reticulocytes rise to higher levels than if smaller amounts are fed. If a large amount is fed in a very short time, the response will come

20. Minot, G. R.; Cohn, E. J.; Murphy, W. P., and Lawson, H. A.: Treatment of Pernicious Anemia with Liver Extract, Am. J. M. Sc. **175**:599 (May) 1928. Cohn, Minot, Alles and Salter (footnote 5).

sooner and will be higher than if the same amount is divided into small doses which are fed over a long time. Further studies on the nature of reticulocytes in pernicious anemia under such varying conditions of response have been made and will be reported in a separate communication. Some of the results of these studies throw an interesting light on the problem of the life of reticulocytes and will be summarized here.

Varying grades of responses have been obtained in this clinic by the administration of experimental fractions of liver extract or of material the potency of which was to be tested. In a series of sixteen cases, in which the initial red blood cell level was between 1,000,000 and 2,000,000 per cubic millimeter, the reticulocytes were studied especially in relation to the amounts of reticular substance which they contained, or, in other words, in relation to their age. It was found that the reticulocytes in those cases which had a maximal response tended

TABLE 6.—*The Three Types of Reticulocytes in the Blood of Patients with Pernicious Anemia Two Days Before the Peak of the Reticulocyte Response to Liver Therapy: Maximal and Submaximal Responses*

Number of Cases	Percentage of Maximal Reticulocyte Response	Average Percentages of Cells Containing Reticular Substance		
		Cells with Large Amount	Cells with Medium Amount	Cells with Small Amount
4	17 to 45	7.0	58.4	34.6
4	100	20.8	58.4	20.8

to be of a considerably younger variety than those which had a submaximal response. This was especially true of the reticulocytes present two days before the peak and at the peak of the reticulocyte response. Table 6 gives the average figures of four cases which had a maximal response, and of four other cases which had a response of less than half the maximal. The maximal response for each erythrocyte concentration was estimated from data given by Minot and Cohn and their collaborators.[20]

In one case in which a submaximal response was first obtained, and was followed later by a maximal response due to the feeding of a more potent material, the reticulocytes during the first response were more mature than those during the second response.

One case (that reported in table 1, experiments 4, 5 and 6) has been studied from many different angles and is of especial interest. This case, in which a maximal response was obtained, showed a prompt reticulocyte rise to a peak followed by a rapid drop to a low percentage in four or five days. The reticulocytes were studied in vitro at 37 C. by the usual technic. The rate of decrease in the absolute number

of reticulocytes in vitro corresponded remarkably closely to the rate of decrease which was observed in the patient. In other cases, however, the rate of decrease of reticulocytes was usually slower, but never faster, than was to be expected from the rate of decrease that was observed in vitro. This may be due to the fact that a small concentration of new, relatively mature reticulocytes enters the circulation following the peak of the reticulocyte responses.

In the same case the total number of new adult red blood cells which were present in the circulation one day following the peak of the reticulocyte response bore a close relation to the number of new adult red blood cells which could have been formed from maturing reticulocytes as determined by the experiments in vitro.

These observations are evidence that the life of reticulocytes in the circulation of patients with pernicious anemia is of a similar duration to the life of these young cells in vitro.

SUMMARY AND COMMENT

It has been shown that reticulocytes exist when incubated at 37 C. for from one to about five days. They remain for a very much longer time when kept in the icebox or at room temperature. That the reticulocytes lose their reticular substance and become adult erythrocytes is shown by the fact that the total number of red cells in the incubated specimens of blood does not diminish as the reticulocytes disappear. Evidence is presented to show that this must be the case in the blood stream, at least of patients with pernicious anemia at the time of the reticulocyte response following liver therapy.

It is never possible to do more than to assume that there is a direct analogy between in vitro and in vivo phenomena. By placing reticulocytes in the pleural cavity of rabbits, by the transfusion of reticulocytes and by observations on patients, an attempt has been made to reach conclusions based as nearly as possible on physiologic conditions. Whether the maturation of reticulocytes in the blood stream is the same as the maturation which takes place normally in great part in the bone-marrow is difficult to determine. A few reticulocytes are found in the circulation in health, and at birth considerable numbers are often present. It would be peculiar if they were destroyed as fast as they entered the circulation, when it is known that other cells of the bone-marrow—myeloblasts and myelocytes—may continue their growth in the circulation.[21]

21. Sabin, F. R.; Austrian, C. R.; Cunningham, R. S., and Doan, C. A.: Studies on the Maturation of Myeloblasts into Myelocytes and on Amitotic Cell Division in the Peripheral Blood in Subacute Myeloblastic Leukemia, J. Exper. Med. **40**:845 (Dec.) 1924.

It has been demonstrated that reticulocytes have a definite oxygen consumption whereas adult erythrocytes have only a very slight oxygen consumption.[22] There is, then, perhaps a direct analogy between the maturation rate of reticulocytes and the rate of senescence or death curve typical of many simple biologic and chemical phenomena. The development of the reticulocyte into the adult nonreticulated erythrocyte may be a true senescence or death. In favor of this is Denecke's [23] suggestion that the juvenile erythrocyte is not such a useful carrier of oxygen as the adult erythrocyte since it requires oxygen itself.

A problem of additional interest is the disposal of the basophilic substance which has disappeared from the young cell in the course of its maturation. It is probable that the basophilic substance diminishes gradually in amount as the cell reaches maturity. The observation of the increase of granules in erythrocytes with the decrease of reticulocytes suggests that this is a normal occurrence in the maturation of reticulocytes. The consumption of oxygen by the reticulocytes leads to the possibility of the disposal of the basophilic substance by normal catabolic processes.

It would be impossible to state definitely the duration of life of the reticulocytes in any one specimen of blood because of the many different ages represented. Reticulocytes containing large amounts of reticular substance probably mature into adult erythrocytes within from four to six days, whereas those containing small amounts of reticular substance probably mature into adult erythrocytes within from a few hours to several days. At all events, it seems evident that progressive changes toward senescence occur in the circulating reticulocytes.

CONCLUSIONS

1. Reticulocytes in vitro at 37 C. and in the pleural cavity of the rabbit decrease at a regular rate over a period of from one to four days. This rate is analogous to a death rate or a maturation rate.

2. The rate is similar for reticulocytes from various sources; for example, from bled rabbits, from those into which phenylhydrazine is injected and from cases of hemolytic jaundice and pernicious anemia.

3. The rate is much slower at 23 C. or at 10 C. than at 37 C. Reticulocytes have been found in blood kept in the icebox for six months.

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22. Harrop, G. A., Jr.: The Oxygen Consumption of Human Erythrocytes, Arch. Int. Med. **23**:745 (June) 1919. Harrop, G. A., Jr., and Barron, E. S. G.: Studies on Blood Cell Metabolism: I. The Effect of Methylene Blue and Other Dyes upon the Oxygen Consumption of Mammalian and Avian Erythrocytes, J. Exper. Med. **48**:207 (Aug.) 1928.

23. Denecke, G.: Ueber den Stoffwechsel der Erythrozyten im anämischen Blute, Deutsche med. Wchnschr. **52**:280, 1926.

4. The decrease in the number of reticulocytes is not related to degenerative changes which may take place in the blood in vitro.

5. No dependable conclusion regarding the maturation rate of reticulocytes can be drawn from the experiments on the transfusion of reticulocytes into rabbits, but the results are not inconsistent with the observations concerning their decrease in the test tube.

6. As the reticulocytes decrease in number in vitro, cells with "granules" progressively increase.

7. Reticulocytes having large amounts of reticular substance are less mature than those having small amounts of reticular substance and require a longer period of time to reach maturity.

8. There is an undoubted analogy between the rate of decrease in the number of reticulocytes in vitro and of those in the blood stream. In both instances this is probably true maturation. Evidence strongly in favor of this conclusion is given by studies on reticulocytes from the blood of patients with pernicious anemia during the reticulocyte responses following liver therapy.

Book Reviews

THE CHEST. By L. R. SANTE, M.D. Price, $20. Pp. 500. New York: Paul B. Hoeber, Inc., 1930.

Under the caption "The Chest" the author has presented in semiatlas form the whole story of pulmonary disease from a roentgen standpoint. To the medical man not experienced in roentgen diagnosis its 500 pages of highly illustrated material may be used as an inductive study course which will stand the test of future experience. The experienced roentgenologist will find it an excellently classified review. The point of view of the author is excellent.

About 150 pages are first spent in describing and illustrating the physical basis for recognizing disease of the lungs by the roentgen rays. The following 250 pages make application of these principles to the great mass of pulmonary conditions most frequently encountered. The final 100 pages deal with the more rare or miscellaneous condition met with in the lungs, pleura, mediastinum and diaphragm.

The book is well balanced in that the major stress is placed on the commoner pulmonary inflammations, whereas only minor attention is paid to the unimportant and the bizarre.

PRAKTISCHE DIFFERENTIALDIAGNOSTIK FÜR AERZTE UND STUDIERENDE. HERAUSGEGEBEN VON PROFESSOR DR. GEORG HONIGMANN. Band III: Differentialdiagnostik in der Psychiatrie, von Hermann Haymann. Mit einem Beitrag: Die diagnostiche Bedeutung der Intelligenzprufungen, von Erich Stern. Price, 17 marks. Dresden, Theodor Steinkopff, 1930.

This work is a brief discussion of the important considerations in psychiatric differential diagnosis. Owing to the size of the book, each individual subject must necessarily be considered briefly. The contents are well organized and easily accessible through an adequate index. The same volume contains considerable worth while material in regard to intelligence tests.

The book is written and constructed for the use of general practitioners rather than for specialists in psychiatry, and seems to fulfil its purpose adequately.

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